SPECIAL INQUIRY

THE HONOURABLE THOMAS BATHURST AC KC

SIXTH DAY: THURSDAY 16 FEBRUARY 2023

INQUIRY INTO THE CONVICTIONS OF KATHLEEN MEGAN FOLBIGG

<EDWIN PHILIP ENFIELD KIRK, AFFIRMED(10.16AM)

<EXAMINATION BY MS ROY

Q. Professor, can you tell us your full name for the record?
A. Edwin Philip Enfield Kirk.

Q. And your work address?
A. I work in two places: At Sydney Children's Hospital in the Department of Clinical Genetics there and at the New South Wales Health Pathology Randwick Genomics Laboratory.

Q. You're a medical doctor?
A. I am.

Q. You have a PhD in Cardiac Genetics?
A. Yes, in congenital heart disease.

Q. You also have some additional training in paediatrics?
A. Yes, so I commenced my training in paediatrics and then specialised in genetics.

Q. As you have told us, you are a Clinical Geneticist at Sydney Children's Hospital?
A. That's right.

Q. And a Genetic Pathologist for New South Wales Health Pathology?
A. Yes.

Q. You are also a Professor of Medicine and Health in the School of Clinical Medicine at the University of New South Wales where you are a conjoint appointee?
A. That's right, yeah. I mean, the appointment is not specifically in Health or whatever; it's just a generic appointment, yeah.

Q. Just a few weeks ago, you were also appointed a Member of the Order of Australia for your significant service to genetic pathology and clinical genetics and to medical research?
A. That's correct.

Q. Congratulations.
A. Thank you very much.

Q. You have authored or co-authored more than 125 scientific publications?
A. Yes.

Q. You are also the author of the book "The Genes That Make Us: Human Stories from a Revolution in Medicine"?
A. Yes.

Q. Which, usefully for those of us at the bar table, explains the field of genetics and genetic discovery to a lay audience?
A. That's the intention.

Q. In your own words, can you explain to us what you consider are the areas of specialised knowledge and expertise that you draw upon to express the opinions that you have in your reports to this Inquiry?
A. Yeah, so as you mentioned, I've been involved in cardiac genetics for a long time. Although my PhD was in congenital heart disease, the field of cardiac genetics in Australia is not very large, and so I have been part of that for a long time and attend scientific meetings locally and so on. From a research point of view, my research has not involved direct research into cardiomyopathies or Long QT Syndrome and so on. In a clinical sense, I do see patients. Although I'm based at Sydney Children's Hospital, as a Clinical Geneticist, we do see adults as well. So I do see patients with cardiomyopathies and Long QT and so on. Not very large numbers; I wouldn't see the volumes that many of the people who are giving evidence do, but I have got quite a long exposure to that. From a laboratory point of view, I have been involved in classifying and reporting cardiac genetic variants since about 2016, and it's been a part of my practice over that period. Again, not very large volumes but it's a respectable proportion of what I do and more recently, it's been something that's become an increasing part of my workload.

Q. Would you say that gives you expertise in translating laboratory results into diagnostic--
A. Yeah, I guess, integrating the two. Yeah, so the advantage I've got having both the clinical and laboratory hats is that - only sometimes it might be a disadvantage as well - I can see things from both perspectives. It perhaps introduces biases but it also is a strength, I think.

Q. In what way might that introduce bias?
A. Well, so for example, in the laboratory there is a fairly hard rule that I'm not allowed to do complex reporting on my own patients, because if I refer a test, I may have a particular question in mind and there might be something that I'm not expecting in the data that I might dismiss inappropriately. Whereas someone who is taking a more neutral stance might not do that.

Q. In the interests of scientific and clinical rigor, you separate those roles?
A. Yep. I think it's hard to think separate. You're thinking, I guess, more trying to integrate them.

Q. You integrate them but sorry, you said you don't review your own complex cases?
A. Yeah, yeah, yeah, that's right, yeah, in terms of my workload, I don't review my own complex cases, yep.

Q. What about specifically, have you undertaken any research or work in the field of electrophysiology?
A. No.

Q. What about specifically in relation to calmodulin or calmodulinopathies?
A. No, I would not consider myself an expert specifically in that.

ROY: Actually, for the record, your Honour, we have obtained an updated CV for Professor Kirk. That has been circulated to the parties and with your leave, it will join the tender bundle at Exhibit 7-04; it will be slotted in behind the reports.

JUDICIAL OFFICER: Thank you.

EXHIBIT #7-04 SUPPLEMENTED WITH THE ADDITION OF UPDATED CV FOR PROFESSOR EDWIN KIRK, ADMITTED WITHOUT OBJECTION

ROY

Q. Professor, you first became involved in this case when those assisting the 2019 Inquiry approached you and requested that you investigate the Folbigg genome?
A. Yes.

Q. Genomes, I should say. In that Inquiry, you were a member of one of two expert groups who each identified the CALM2-G114R variant in Kathleen, Sarah and Laura Folbigg?
A. Yes.

Q. You provided three reports to that Inquiry which, for the record, are the joint report of the Sydney Genetics Team dated 29 March 2019, which is Exhibit 2-Z at red page 5205, I'm not turning it up, the second is the response from Professors Kirk and Buckley to the joint expert report of Professors Vinuesa and Cook, which is Exhibit 2-AX, red page 6898, and the supplementary report of Professors Skinner, Kirk and Buckley dated 5 July 2019, which is Exhibit 2-BV, which is at red page 7859. Apart from the red page numbers and the exhibit numbers, does that sound right?
A. Yes.

Q. For this Inquiry, at the request of those assisting his Honour, you've authored or co-authored two reports. The first is the report for the Inquiry into the convictions of Kathleen Megan Folbigg dated 31 October 2022 which is found at tender bundle 2, Exhibit 7-03, red page 47. With that report there were two addendums?
A. Mm-hmm.

Q. There was a response from Professor Kirk and Professor Skinner to questions set on 6 October 2022. That response from you is dated 1 November 2022, that was provided in supplement to the first report where you answered specific questions, yes, that's found at Exhibit 7-03, page 62, and second there was a short addendum to that report regarding the KCNAB2 variant dated 9 November 2022 and that is found in Exhibit 7-03 at page 68-1, is that right?
A. Yes.

Q. The second and final report you completed is the responses from Professor Kirk to the transcript of oral evidence of Professors Toft Overgaard and Nyegaard dated 15 January 2023, is that right?
A. Yes.

Q. For the record that's found at Exhibit 7-04, page 71. Your first report together with the addendums were co-authored by you together with Professor Skinner?
A. Yes.

Q. Professor Skinner is a Paediatric Cardiologist and Electrophysiologist at Sydney Children's Hospital?
A. Yes.

Q. He's a clinical Professor at the University of Sydney?
A. I believe so, yep.

Q. He is an Honorary Professor at the University of Auckland?
A. That sounds right, he worked in New Zealand for a long time.

Q. In relation to Professor Skinner, are you aware that for reasons beyond his control and unconnected with the subject matter of this Inquiry he has been unable to give oral evidence to the Inquiry?
A. Yes, I'm aware of that.

Q. Diagnostics is a primary part of your work as a Clinical Geneticist?
A. Yes.

Q. How do you go about diagnosing a genetic condition?
A. That's a very broad question.

Q. It is.
A. So genetics is a branch of medicine and like all of medicine we rely on the medical history, clinical examination and investigatons. In genetics we would include the family history which should be part of everyone's practice but obviously is a particular focus of ours.

Q. Why is it a focus of yours?
A. Because we are often dealing with conditions that may have a familial component and knowledge of the person in front of you may not give you complete information. Sometimes it's putting together information from the

wider family that gives you the diagnosis. Sorry, want me to elaborate on?

Q. Please?
A. As I said, it's a broad question, but the - so I talked about the family history--

Q. Could you perhaps address it this way, what are the sources of information that you are drawn to to diagnose a genetic condition?
A. Right. So, textbooks are becoming less and less a thing. There are online databases, the medical literature directly and our ability to research the medical literature is obviously a lot better now than when I first started. So - and then if we are considering for example the pathogenicity of a variant, there are lots of sources of information that we can potentially draw on such as population databases that supplement the information from the medical literature.

Q. You're doing that having started with a patient in front of you?
A. Yes.

Q. You mentioned notwithstanding you were at the children's hospital you also see adults, in what capacity?
A. So, because clinical genetics encompasses the whole lifespan, limiting a practice to just children is kind of artificial and not realistic. So, I see adults in two main contexts. Firstly, where there's a direct question about that person themselves, for example, someone has clinical evidence of Long QT Syndrome and there's a question about genetic testing in that person, or where there's a family history and there's a predictive test being considered where there's a known pathogenic variant in the family, and then I also see adults in the context of pregnancies. So, for example, I see patients at the Royal Hospital for Women who usually are already pregnant and there's been say a finding at ultrasound that raises the question of a genetic condition. Those would be the main capacities in which I see people for themselves, but when I'm seeing a child, that may also involve seeing the adult. So I will often, reasonably often, examine parents as part of my assessment of the overall situation and occasionally, not very frequently, will make a diagnosis of a syndrome in a parent that they were not previously aware of because we've made that diagnosis in the child.

Q. I want to come to the ACMG Guidelines.
A. Yep.

Q. You very eloquently explained how they operate in your first report--
A. Thank you.

Q. --so I won't ask you to step us through that, but I just want to supplement that information. You note in your first report, we can turn it up if we need to but--

JUDICIAL OFFICER: That is the first report to this Inquiry.

ROY: In this Inquiry, thank you, your Honour, which is Exhibit 7-03 at red page 47.

Q. You note that there's a major revision to the classification system under development?
A. Yes.

Q. To move towards a probabilistic framework?
A. Yes.

Q. Can you explain that?
A. Yeah, so when we classify variants, we've got this five-level system from benign, likely benign, variant of uncertain significance, likely pathogenic and pathogenic. Those are categorical divisions, and it obviously can make a very big difference whether something sits at variant of uncertain significance or likely pathogenic and a lot of our time in difficult classifications goes into where things sit on that borderline. But the underlying evidence that we are dealing with essentially combines in a probabilistic way. So, how likely is it that, for example, a variant that's present in one in ten people would be pathogenic, and the answer is very unlikely. How much weight can we put on the information that a variant is absent from a large population database, and so there have been efforts made to work out what the weighting, the probabilistic weighting of each of those things is, and I don't know if you want me to explain Bayesian probability theory--

Q. No, thank you.
A. --but the idea is to try to integrate information in a semi-quantitative way so that you come up with a score, and part of the reason for that is that variants of uncertain significance covers a multitude of different possibilities. So, it includes variants where we've sweated and stayed up all night trying to decide whether something should be classified as a VUS or likely pathogenic where clearly one piece of information might tip it over the line through to variants that really we don't think anything very much of, like the KCNAB2 variant, but we haven't got strong enough evidence to call it likely benign or benign. So by having a points basis, the idea is that for the person - so the idea is the person reading the report will have a clear idea of how that information was put together and exactly how close the call was.

Q. Is that to say at the minute a report might say a variant of uncertain significance tells the reader of the report there's somewhere between a ten and 90% chance that the variant is pathogenic?
A. In principle, although really most variants--

JUDICIAL OFFICER

Q. Is it done by a score? How does it work? Sorry, I don't want to go too much into probability theories, but you mentioned the probabilities by reference to what?
A. Right. So there's a paper - the first author has just escaped me - from a few years ago where a group who are very active in this space, Tavtigian et al.,

were a group that were very active in this space, took the ACMG criteria and measured the information that was provided by the application of each criterion against a set of known data to work out the relative contribution of each of them, and they found that the levels that had been generated, essentially by experts who had joint opinions, corresponded pretty well to the amount of weight that the criteria tended to add to the likelihood that a variant was pathogenic. I think there's a risk of assuming that something is more precise than it really is, so if we say something's 90% likely, that's an approximation and the numbers that have gone into that are not hard and fast.

Q. So there's a very significant margin of error between, putting it crudely, what's on the borderline of likely pathogenic and VUS?
A. Yes, there's definitely a margin of error, and that's part of the reason that even by applying the same criteria different experts using the same information may come up with a different answer. There's still an element of judgment, and I think that's not going to go away in the near future. Some things are objective, is something in a database or not, but the weight you put on some other types of information still requires subjective judgment. Part of the issue with this Bayesian analysis, and I'm not going to go into it, starts with what the prior probability is that someone's got a pathogenic variant in a particular gene. So, if someone's got a family history of breast cancer, they had breast cancer when they were 30, their mother had breast cancer when they were 30, there's two or three other people with early onset breast cancer, the prior probability that that person has a variant in one of the known breast cancer genes is really quite high. Whereas if you take someone from the general population with no family history, the prior probability is quite low. And so that influences the way that you think about the information, and that's probably - no, I think that is relevant to this case because we're assessing a variant in an individual who, particularly an individual who at the moment doesn't have a clear-cut clinical diagnosis.

ROY

Q. You're referring to Kathleen Folbigg?
A. Yes.

Q. How would the prior probabilities work in this case, how would you weigh that?
A. Well, we're not currently using a system that spits out a number, but--

Q. Yes. Accepting that, yes?
A. So I guess really it's - the normal situation is that we've got a person with a phenotype, we do a test and we interpret those bits of information together. In the case of Kathleen Folbigg I think there is some question about whether she has a phenotype. In particular, Professor Wilde interpreted the exercise test as potentially showing some features either of CPVT or Long QT Syndrome. As I read his report he was not making a diagnosis, he was just saying there are some features that could be consistent but are not diagnostic. None of the other cardiologists who looked at the same data have come to the same conclusion. Arthur Wilde is a really, really good cardiologist and knows the

stuff as well as anybody, so I, you know, I don't know what to do with that, but if you accept, for example, a clear-cut that this person's got, that Kathleen Folbigg does have one of those conditions, then that has a profound influence on the way you think about classifying the variant. Whereas if you say, look, we don't know if she's got a relevant phenotype, then it puts you in a different category from the starting point in classifying the variant.

Q. What about, I think it was Professor Watkins? I might turn up his report which is volume 14, tab 10 and we'll put it on the screen. It can be given to you in hard copy but it will also come up on the screen.
A. Yep.

Q. On page 2 which is red page 322 of that report he says, this first dot list he's explaining why he concludes the variant is likely pathogenic.
A. Yep.

Q. The second black dot point, I want to understand if he's making a similar point but in relation to a different factor?
A. Mm-hmm.

Q.

> "The genome sequencing was done to look for a possible genetic explanation for sudden death in the Folbigg infants. I believe that the *a priori* chance that a pathogenic or likely pathogenic variant would be identified in one of the small number of genes associated with sudden death under the age of two is low."

A. Mm-hmm.

Q. Just pausing there, do you agree with that?
A. That a pathogenic or likely pathogenic - yes, I agree with that.

Q. Yes?
A. Yep.

Q. Is that a similar factor that could weigh in an analysis as to pathogenicity?
A. I think he's taking it the other way.

Q. Yes.
A. He's saying it's unlikely that just by chance, you would find a likely pathogenic or pathogenic variant. So I think what he's saying is that if you classify the variant as likely pathogenic or pathogenic then that's unlikely to represent a coincidence.

Q. Can that--
A. That's how I interpret that sentence.

Q. Can that be used as a factor in favour of pathogenicity?
A. No, no, he's stating it from the other direction, I think.

Q. He states it in the other direction, is it?
A. But that's how I read what he's - what he's saying there, yeah. That if you've got a pathogenic or likely-pathogenic variant in the context of sudden deaths, it is unlikely to be a coincidence. I think that's what he is saying.

Q. Quite, yes, so does that - maybe we're at cross purposes. If it is unlikely to be a coincidence that you'd looked for a variant because you have a suspicion of sudden unexpected death, sudden unexplained death, and then you find one--
A. Oh I see, right. I guess the problem is that there are multiple potential causes of a sudden unexplained death. There have been lots of potential causes for the deaths of the four children suggested. Clearly, even in this family there are two children who definitively did not die due to this calmodulin variant. So I mean I guess in that context - I'm not quite sure I'm answering the question.

Q. Maybe I'll do it this way: You've described in your report the risk of circular reasoning.
A. Yes.

Q. Reasoning from the fact of the girls' deaths.
A. Yeah.

Q. What Professor Watkins is doing there, to be fair, is not referring to pathogenicity but to the likelihood that it was causal in relation to the girls' deaths.
A. Yeah, if you classify it as pathogenic or likely pathogenic, yep.

Q. You can't use that reasoning to contribute to a finding of pathogenicity?
A. No, I don't think so.

Q. Because it would be circular?
A. That's right.

Q. You have also raised in your first report in this Inquiry the question of whether the guidelines can be an appropriate standard against which to assess the potential pathogenicity of G114R for the purposes of this Inquiry?
A. Yes.

Q. What did you mean?
A. The - the standards that we use in diagnostic testing are really quite stringent. The reason for that is that we've made, as a field, lots of mistakes in the past. We used to get it wrong with frightening frequency. The reason we know that is because of these population databases that have come along. So as a result, the field has become very cautious about how we classify variants and I think that's appropriate because the classification we give to a variant has consequences for patients. It's got consequences in both directions:

If we wrongly classify a variant as pathogenic or likely pathogenic then, for example, that might mean that someone terminates a pregnancy based on that

information, or has a bilateral mastectomy or whatever it is depending on the clinical circumstances.

On the other hand, if we wrongly classify a variant as a VUS when it should be classified as a likely-pathogenic variant then that can also have consequences. Someone who should have the opportunity to have a bilateral mastectomy for their risk of breast cancer may not and they may develop breast cancer and so on. So because of that - that concern for not doing harm, there's been a relatively stringent standard set and it's definitely informed by the realisation that many of the classifications that we - that we generated before the population databases came along were wrong, and that's particularly true in the field of cardiac genetics but by no means restricted to it. That's a standard that has been set for particular reasons.

Now, I don't know whether that's the same as the standard that you would apply in terms of a legal case. It might be that a lesser level of evidence would be sufficient to make a decision; that's obviously outside my area of expertise.

Q. Is it the case that it would never be valid in the context of clinical decision-making to have regard to a variant of uncertain significance?
A. It depends what you mean by "valid".

JUDICIAL OFFICER

Q. Let me ask you it this way:
A. Yep.

Q. In considering the possibility of a death being due to a genetic mutation--
A. Yep.

Q. And I emphasise the word "possibility".
A. Yes.

Q. Would it be appropriate in your view to look at, investigate, a mutation that has been classified VUS?
A. Would it be appropriate to investigate it?

Q. Yes, you have a person who's died.
A. Yep.

Q. You don't - you can't identify a clinical cause.
A. Yes.

Q. You know she has a variant--
A. Yep.

Q. --which has been classified as a variant of uncertain significance.
A. Yes.

Q. Would you dismiss that variant, absent any other evidence, as a possible

reason for the sudden death?
A. Oh goodness, no, no. Uncertainty means exactly that; it means we're uncertain, and our degree of uncertainty, as I said before, varies. No; to classify a variant as a VUS is by no means to dismiss it as a possible cause.

Q. As a database expands--
A. Yep.

Q. --showing deaths with persons who have that mutation without an explanation--
A. Yep.

Q. --the classification will move presumably?
A. So yeah, there are two things that I think potentially could move the classification of this variant: One would be if we had a definitively affected person who had the same variant; I think that would definitely tip it towards or over the line to likely pathogenic. And the other would be if we started getting more individuals in, as population databases grow, and it became apparent that the variant were more common than we would expect for a very rare pathogenic variant.

Q. I put that proposition to you on the basis of totally unexplained clinical reasons for the death.
A. Yep.

Q. If you get to a position where people have that particular mutation and there's a variety of what I'll call "clinical causes" of death--
A. Yes.

Q. --that would instil, influence consideration of whether the mutation was pathogenic or not, presumably?
A. I'm not sure I understood that question, I'm sorry.

Q. Probably because it was very badly put. A number of people with the mutation have died.
A. Yep.

Q. But the particular cause of death for each of them is different.
A. Yes.

Q. How would the significance of the mutation be considered in that context?
A. Okay. So if I knew that there were other potential causes of death, that would reduce my confidence that the variant was likely pathogenic. Because, if you're classifying a variant as a variant of uncertain significance, you're essentially saying "I don't know what caused this death" in this context, but if I do know what caused this death, then - then that really reduces the likelihood that there's an alternate cause of death. So yeah, I think I see what you're saying now. So yes, the level of evidence in relation to other potential causes such as myocarditis, for instance, is, to some extent, relevant to how we think about the pathogenicity of this variant.

ROY

Q. I think in a similar vein, can I ask you a hypothetical?
A. Sorry, say again?

Q. I'll ask you a hypothetical scenario?
A. Yep.

Q. Assuming this family came to see you in clinic, putting to one side the spectre of criminality, you just have everything you know about this case to date.
A. Yes.

Q. And they were considering attempting to have a fifth child.
A. Yep.

Q. Would you encourage them or discuss with them screening their embryos for this variant G114R?
A. I mean, part of the problem is that I would know that it wasn't going to address two of the deaths, which may have an alternate genetic cause. Strictly speaking, if you classify a variant as a VUS, that implies that you are assessing it as not being clinically actionable, and we're mostly fairly strict about applying that rule. Very occasionally, there are exceptions made. So I can't say I would never consider that. In the context of two additional deaths, I would be very worried about it because I would know I was only addressing part - at best, addressing part of the problem, and also that I might be selecting embryos the wrong way. I might be choosing embryos that are affected but thinking that I was - that I was doing the opposite. Very occasionally, if there is a variant that's got, you know, not quite enough evidence to get to likely pathogenic, there's no alternate explanation - and I think that no-alternate explanation is probably quite important for the phenotype in the child - you would - you might consider doing that. Laboratories seem to be quite reluctant to do the testing because that's the view of the field, that you shouldn't be doing that, but it occasionally happens. I can't remember that I personally have ever done this, but I've had colleagues who have done it and - and I have supported the decision in that - in a couple of instances.

Q. Having regard to the unusual features of this case, including the death of the two boys, should I take it that that puts this variant, for you, in the category that you would be sweating, up at night, trying to work out which category it fits into?
A. No, this is a fairly straightforward classification from my point of view.

Q. You wouldn't be troubled by not including it in a screening?
A. Hmm, would I be troubled? No, I might be troubled, actually, yeah. I mean look, there's evidence in both directions and - and I can't say for certain that it's benign and if it's not benign, the consequences of that, you know, are bad. No, I think I would be a bit troubled by it, yeah. I didn't find it a difficult classification but that's not the same as saying I wouldn't be worried in that

scenario; I think I would, hmm.

Q. Would you then encourage screening or not encourage screening?
A. I wouldn't be encouraging it.

Q. You wouldn't be encouraging screening?
A. No, but if the question was raised.

Q. As in, raised by the parents, by the family?
A. By the family, yeah. I would certainly be willing to engage in the discussion.

Q. Within the 10% to 90% range - not applying the guidelines but applying your own judgment - can you place the variant anywhere?
A. I don't know that I can, to be honest. Yeah, I'm sorry. It's - I've thought about that question quite a lot and I don't have a decent answer. But I should also say that that 10% to 90% range is very notional at the bottom end. I don't think this is at the bottom end.

Q. What does that mean?
A. Well, everyone has many, many variants that, if you classified them, would come out at a 10% chance of being pathogenic. So the system breaks down, and that partly relates to the way that those numbers were generated, which was in a context of someone who had a high chance of having a pathogenic variant and there were ten variants found. So it starts at a presumption that there's about a 10% chance that anything you find is relevant. That's - that's from the Tavtigian paper, yep. Whereas in the real world, everybody has many, many variants that could potentially be classified in that 10% range, but that's not really meaningful.

Q. If we go back to Professor Watkins - I called it a "report" but it's actually a letter, which is volume 14, tab 10, red page 322. We were looking at the first half of what he said in the second black dot point.
A. Yep.

Q. Coming to the second black dot point, he was referring to the *a priori* chance and he concludes "In a routine clinical setting, I would absolutely conclude that a variant in this gene with these characteristics was the likely explanation following a young sudden death with a negative autopsy".
A. Mm-hmm.

Q. He emphasises "In a routine clinical setting"; do you agree or disagree with that?
A. I don't think that I can fully agree with that. I would say that there is room for uncertainty. We know that there are individuals in the general population who have variants in genes like this. So I asked one of our biomathematicians to have a look at a small database that we have got, which has only got a few thousand people in it so nothing like the - the gnomAD database, and there were three individuals with missense variants in one of the CALM genes that were absent from gnomAD or only had one individual out of a few thousand. I

haven't gone to check that all of those are real variants; it's possible that they are false calls by the software - except for one that's in two related people that I'm sure is real. I haven't assessed the likelihood that those are pathogenic variants, but they're not people who've got relevant phenotypes. So clearly, that's a small percentage, but that's only three genes and there are plenty of other genes in which you could have found a variant that might be relevant; if you have Long QT genes and so on. So you know, you clearly can find a rare variant that, in a gene that's relevant, and it might not be pathogenic. That's a possibility. So, I think I would view that a little differently.

Q. Is that then from - aside from what you've just said, we've otherwise heard figures by reference to the gnomAD database, which - and you refer to verifying whether there were actual variants or not?
A. Yep.

Q. The verified numbers in the gnomAD database across all three calmodulin variants. It hovers around 30, I think it's either side of 30?
A. Yes, it's certainly very small.

Q. And around about 10 per each variant?
A. Yep.

Q. And somewhere I think between 6 and 11. Does that, do the numbers in your database of 4,000--
A. Yeah.

Q. Do they correspond to that?
A. Look, the degree of confidence you'd have in such small numbers, I don't think you could be, you know, it might be chance. We might do another 10,000 and not find any.

Q. The G114R variant has been described as an ultra-rare variant by a number of experts in this Inquiry?
A. Mm-hmm.

Q. What's your view of that expression?
A. I'm not a fan of that term, but I guess that's just a language thing really.

Q. Why aren't you a fan of that term?
A. I don't know that it really helps. I'd rather just describe what the numbers are than introduce a term that - it sounds a little bit emotive. The truth is that everybody has variants that have never been reported before, so every time we do a diagnostic exome sequence we see variants that are not in any of the databases. The amount of normal human variation is enormous, and we are not even close to capturing all of what's out there.

Q. But what about coming to calmodulin specifically?
A. Yep.

Q. You would accept that they are amongst the most highly constrained genes

in the human genome?
A. Yeah, they're highly - yeah. Very highly constrained.

Q. That they're generally seen in much lower rates than most other genes - variants - I should say they, variants in calmodulin.
A. Yeah, that's saying the same thing in different ways.

Q. Same thing?
A. Yeah.

Q. Is describing them as ultra-rare just another way of describing that phenomenon?
A. Usually it's used to refer to a specific variant, so people say this variant is ultra-rare rather than variants in these genes are ultra-rare.

Q. I see. Those factors otherwise, the highly constrained, ultra-conserved--
A. Yep.

Q. Do they limit the utility of the guidelines in the application to calmodulin variants generally?
A. Look, I think there's potential that they might and I wouldn't say that I was an expert in the calmodulin genes that I could address that with a high degree of confidence. It's certainly true that we run into clinical situations and diagnostic situations where the ACMG criteria don't capture the most important evidence very well, or you know, there are situations where - so you've seen in the report that I upgrade and downgrade criteria based on my opinion about the strength or weakness of evidence, and there are situations where you might have a particular piece of evidence that outweighs other things in a way that it might not normally.

Q. I take it from that you think the rarity of calmodulin might do that in this case? Or might validly be weighed in that way in this case?
A. I think a reasonable person could take that view. Yes.

Q. But you don't take that view?
A. I don't, but I wouldn't criticise someone else for doing it.

Q. Why don't you take that view?
A. I was trying, when I did this classification, to work with the standards as we have them and with what's known, so when I upgraded or downgraded it was when something was very clearly in my mind, kind of a lineball call, and in fact most of the decisions I made in that - all of the decisions I made in that direction I think were in the direction favouring pathogenicity, but I don't know that I've got a basis on which to interpret the constraint of one gene versus another in a way that I can meaningfully apply. I might be wrong, by the way. I could easily be wrong.

Q. Would you accept Professor Nyegaard is an expert in calmodulin?
A. Yep.

Q. She's certainly taken the view that that's an overwhelming factor in this case?
A. Right. I mean, with respect to Professor Nyegaard, I think her area is more about the cell biology and the functional studies, rather than variant classification and you know, fair enough, I hear that opinion but I don't think it changes the way that I think about it.

Q. Notwithstanding that other experts in this Inquiry, and not limited to Professor Nyegaard, take a different view, you maintain that - I withdraw that. Are you not modifying your view by reference to the rarity of calmodulin because that's an area outside of your expertise or are you not doing it because you feel that it's not appropriate in the case of calmodulin?
A. I'm not doing it because - it certainly, I mean, variant classification is well within my expertise. Why am I not doing it? That's a fair question. I think it's because it's not - it's not something that we usually do. So, you know, I certainly - geez, that's a weak answer, isn't it. I guess it's that I don't have a basis on which to judge when one should do that. It's not something that I've come across in the course of my practice previously, and I don't have a sound basis on which to say at this point we upgrade, so in a lot of other situations it's fairly straightforward or if not straightforward you can say I'm basing it on this, and here, I don't know where the cut off would be. I don't know, you know, at this level of constraint I upgrade; at that level I don't. But look, let's say that we upgraded it to moderate evidence, it wouldn't actually change the classification of the variant and I don't think I would go as far as strong.

Q. If it upgraded to moderate it wouldn't change the variant classification?
A. No. No.

Q. Is that because of the weighting the guidelines give to this factor generally or?
A. It's more about would it be enough to offset the fact that we don't have a clear phenotype.

Q. Okay, I might come back to that. What about in respect of this variant in particular, G114R, and we've already commented a bit about the limits of the phenotype, the phenotypic evidence that you have?
A. Yep.

Q. So, the children are deceased, neither Sarah nor Laura had the 12-lead ECG?
A. Yeah.

Q. That in your report, and I assume Professor Skinner contributed significantly on that front?
A. Entirely, yes.

Q. That would be, yes, desirable to have, to record, the heart function. Laura only had a single ECG taken during a sleep study and then we have that Ms Folbigg is the only other accessible carrier. First of all, do you agree that there is limited information about the phenotype of the three known carriers?

A. Yes. Well, four. We've got - yeah.

Q. My next question, do you include the fact that there is a fourth known carrier in CALM3 that was identified?
A. Yes. Well, yes and no. I did not take out the absent from population databases criterion. I guess you could, you know, because strictly it says absent, and the variant's not absent, and also thanks to Professors Nyegaard and Toft Overgaard we know that this person's in the UK Biobank, they're certainly the same person in the UK Biobank and doesn't have any indication of heart problems. People in the UK Biobank get quite a working over but not to the level that would be required to diagnose a subtle phenotype, so you've got two apparently healthy adults with the variant. But, even so, that's one out of a very large number, I was considering that you could have a non-penetrant individual although the evidence for that prior to Professor Schwartz's recent report was quite limited. So, I chose not to take that criterion out. Obviously it's in my mind, that we've got the additional individual who's healthy or apparently healthy.

Q. Putting how you've actually applied it in this case to one side, the fact of the limited evidence, does that limit the efficacy of using the guidelines in a meaningful way?
A. No, we always have some limitations on the evidence available to us, so there are all these different categories of evidence that you might use for assessing a variant and it's very rare that we've got something in every category. It's the rule that we operate on a set of evidence that is a subset of all the possible evidence you might have.

Q. So, would it be correct to say that if anything, the guidelines serve to reinforce the importance of evidence and if you don't have the evidence you end up with uncertainty? Is it as simple as that?
A. Yeah, I suppose. I mean that's more the principles on which they're founded, is about putting the available evidence together and if you haven't got evidence then you've got a problem.

Q. You were mentioning that you're not sure how to deal to a certain extent with the extent of the constraint in this case and how to--
A. Yep.

Q. Are you aware of the expert consensus statement on the state of genetic testing for cardiac diseases?
A. I have seen it but I haven't read it. It's quite a long document.

Q. Yes--
A. And fairly recent.

Q. And by “seen it” because I asked you about it this morning?
A. Yes.

Q. Yes. Are you aware generally about expert consensus statements in the field of genetic testing?

A. Yes, they come out fairly regularly.

Q. What's their purpose?
A. To act as guidance for people working in the field, so this one covers a whole range of things, not just about doing a test and interpreting the result, but acting on it as well--

Q. I'll just interrupt you for a second. When the Professor is referring to this one there is a--

I'll just put it on the record, the European Heart Rhythm Association,Heart Rhythm Association Asia Pacific Heart Rhythm Society and the Latin American Heart Rhythm Society's Expert Consensus Statement on the state of genetic testing for cardiac diseases, which was published in Europace in 2022 and is found at Exhibit 15, tab 262, red page 3624. I'm not - I've read it out. I'm not asking everyone to turn it up but for the record.

Q. I'm sorry, so the purpose of statements like this?
A. So, this one, having leafed through it, looks like it covers questions like when to test, which genes to test, what you do with the information once you've got a result. Some guidelines are more limited than that. There are gene specific guidelines for variant classification, for example. So, to the hypertrophic cardiomyopathy genes, MYBPC3 and MYH7, there are documents written about how to classify variants just in those genes that are written by expert groups and so if we're doing variant classifications in those genes we use those modified criteria rather than the standard ACMG criteria as our guide.

Q. Those modified criteria are put together by experts in that particular gene?
A. Yes. In the field - well, anyone working in cardiomyopathy will be familiar with those two genes.

Q. In the particular variants?
A. But yeah. Yep.

Q. Would that be the sort of same guidance in that respect that might give you guidance as to what to do with a very highly constrained gene such as calmodulin--
A. Potentially, yeah. Yeah, I haven't seen specific guidance about whether those should be treated differently--

Q. I'm not suggesting there is one?
A. Okay.

Q. Coming now to, we've dealt with some of it, but coming back through and stepping through your view of pathogenicity in this case.
A. Yeah.

Q. In your report, Exhibit 7, tab 3, red page 54 which is page 8 of your first report. We'll step through these criteria now. You're concluding there an

analysis of the phenotypic information, and you conclude at the top of that page in bold, "It should be noted at this point that it is very difficult to reach a classification of likely pathogenic in relation to a variant associated with an autosomal dominant condition, when clinical information for the only person available to assess is inconclusive"?
A. That's right, yes.

Q. First of all, what is autosomal dominant?
A. So, there are 23 pairs of chromosomes. The X and the Y are the sex chromosomes, and the other chromosomes are the autosomes, and each of us has two copies of each of the autosomes, so a gene that's located on one of chromosomes 1 to 22 is an autosomal gene. Dominant inheritance refers to the situation where having a variant in one of your two copies of that gene is sufficient to cause a - or that may be sufficient to cause a phenotype, and so what happens is that in families a person who has a condition, each time they have a child has a one in two chance of passing it on.

Q. Do I take it from that you're inferring from the available data, which would be the Registry, that this is an autosomal dominant condition?
A. Yes, that's clear-cut.

Q. Yes, and when I say this, calmodulinopathy?
A. Yes.

Q. Yes. Why then is it particular to that type of condition that it's difficult to make the assessment when the only person available is inconclusive?
A. It's true in other - in other forms of inheritance as well.

Q. So it's just generally difficult with limited phenotypic information?
A. Yeah. Yeah, there are some specific circumstances where we might, particularly in carrier screening with the loss-of-function variants in genes where - there are specific circumstances where there might be an exception, but in general we are starting from a phenotype.

Q. You then discuss at number 2.2 on the same page, red page 54, the functional data and how that can be weighed. In the second paragraph you're expressing caution about single cell assays?
A. Mm-hmm.

Q. You conclude that in the diagnostic laboratory setting, this is the third from the bottom line, functional assays are treated with caution because all assays can produce false positive results and research assays are seldom assessed and validated to the same level as diagnostic tests. In the final paragraph on that page, you describe that without having tested benign variants, in this case, there's - and by benign variants I refer to benign calmodulin variants against the same assays - there's no way to determine the likelihood of a false positive result?
A. Yep.

Q. You conclude that if the necessary studies had been performed, this could

be upgraded to moderate - this--
A. Mm.

Q. --the functional criteria. After your report there was some additional work done in the lab with Professor Toft Overgaard that you've seen the report of that work?
A. Yes.

Q. In your second report, which is responding to that work, at Exhibit 7, tab 4 on red page 72, which is the second page of that report, at the top of that page you note the additional work on the I10T variant, which was the deemed benign variant, you say:

> "… it is helpful to know that the assay on which this variant was tested is capable of yielding a negative result in assessing a missense variant that is considered likely to be benign. However, this is not sufficient to validate the test to a diagnostic standard, or to change our interpretation of the variant."

A. Yes.

Q. Those are two separate things, not enough to validate to a diagnostic standard?
A. Yeah.

Q. But also that it did not change your interpretation of the variant?
A. Yep.

Q. Why wasn't it sufficient to change your interpretation of the variant?
A. So this is something where we do have pretty clear guidance, so the ClinGen group have put out a publication which I've referred to somewhere that provides standards by which one assesses functional assays. They particularly warned about being cautious in cells in assays, in over expression assays, in cell systems that are not physiological, so these are kidney cells, embryonic kidney cells rather than heart tissue, but they also lay out how many variants of each type you would need to consider upgrading a criterion to moderate, and one doesn't cut it, you really need about half a dozen. To get to a diagnostic standard you need quite a lot more evidence than that. It's very difficult to validate an assay to the diagnostic standard. In a laboratory setting even a known assay that's well-established, when you bring it into a laboratory you have to do a lot of work to demonstrate it's performing as it's expected to.

Q. Would you ever expect to see a functional assay validated to a diagnostic standard that would be useful in categorising a novel variant?
A. Yeah, potentially, depending on what you mean by a functional assay. So, the example that comes to mind is that there are many - they're called inborn errors of metabolism, where a particular enzyme is deficient, and so an enzyme's a catalyst that helps some chemical reaction occur that wouldn't occur all by itself. There are a number of different conditions where there are inherited deficiencies in enzymes and there are very well-validated assays that

measure enzyme activity. They do have a chance of false positive and false negative results for various technical reasons, but those are at a standard where you could use them as quite strong evidence.

Q. Is that the exception rather than the rule when it comes to - novel variant?
A. I would say so, yes, yeah. There've been many, many different assays that have been developed for research purposes, and generally speaking when you develop, when labs develop these kinds of assays, the intention is not to classify a variant, the intention is to understand the biology. I think I referred to this in one of the documents. The idea is that you observe something that you have evidence is related to the phenotype in an individual and use the assay to try and understand why A leads to B. Some of the assays were developed more to understand the underlying physiology in normal tissues and then have been applied to looking at abnormal situations, but that's the essential difference I think between a purely research assay and a diagnostic assay. Assays can move from one of those to the other, so for example, the enzymatic studies I was talking about I think very likely did move from one to the other. But the great majority of research assays never get validated to a diagnostic standard because it may not be technically possible to do it, or because performing the assay is just inconceivably difficult in the context of a laboratory, all sorts of reasons.

Q. You've said it's very clear under the guidelines that this couldn't change the criteria?
A. Yep.

Q. That a single additional assay in relation to a presumed benign variant, but did it move your own assessment of the value of those assays, in any way?
A. Well, as I say, it's nice to know that the - so it's nice to know that the assay is not just a thing that says this isn't the wild-type, sorry, this isn't the normal version of the protein, that's nice to know, but no, not really. No.

Q. It's otherwise - there are a number of functional assays in this case, we'll come back to some of the others, but Professors Vinuesa, Cook and Arsov had used the expression of stacking, you've got a stacking of functional assays that are all moving in the direction of--
A. Yep.

Q. --the pathogenicity. That doesn't move your opinion?
A. This one, no.

Q. Or the stacking of all of the other ones that are coming up?
A. Yeah, look, I mean potentially - when I looked at what was there, it didn't seem to me that there was anything that accumulatively raised the standard of the evidence, but I'm not an expert in the assays that were used and it's possible that someone who was might take a different view.

Q. If we go back to your first report, which is Exhibit 7, tab 3, at red page 55 you refer to the absence of the variant in population databases?
A. Yep.

Q. This is item 2.3. We've already discussed some aspects of that, I don't want to repeat it.
A. Mm-hmm.

Q. Have you had a chance to review the report of Professor Calum MacRae?
A. Yes.

Q. One of the things he observed is that it cannot be assumed that variants in the same allele in different calmodulin genes will produce the same phenotype.
A. Say that again.

Q. In other words, that CALM1, 2 and 3 are not interchangeable?
A. Yeah. So the evidence that he presents in that statement mainly relates to loss-of-function variants. So he talks about the mice that are different from one another and I guess from that he infers that that might also be true for missense variants. It's not entirely clear why the mice are different, and it may relate to the - so genes are dynamic things, so they're not always switched on or switched off in a given cell, and the extent to which a gene is used can vary during development. It might be, for example that, I think it was CALM3 where the mice just were not born at all, it might be that CALM3 is the really important gene early in embryonic life and the other two only get switched on later on, so that would be a potential explanation for why knocking it out might cause that to happen. We've got some evidence referred to by I can't remember which report, I'm sorry, that the ratios of the RNA that code for the genes in heart tissue are fairly similar for the three genes.

Q. Professor Abrams for the record?
A. Sorry?

Q. I think it was Professor Abrams' report?
A. Right. So, it's likely that there isn't a very big difference in a clinically relevant tissue in a person who has already been born. Because the activity of the variants, the way they work is thought to be activating, when you think about it, if you've got three genes, each being produced, producing the protein at about the same level and you've got a variant, then only one in six of the copies, in which you've got two copies each times three genes, only one in six copies in the cell is going to have that change. You would think that's a relatively small difference if it was talking about loss-of-function, and indeed we're talking mostly about a gain of function or a change of function as the likely mechanism. I would I think disagree with Professor MacRae I think in that I think that it's likely any one of those proteins with a similar change will have a similar effect. There is some clinical evidence for that in that there are people with the same variant in different genes with the same phenotype, yeah, so I don't think I share that view.

Q. You've said you don't share that view, it would seem that that would be relevant in two respects, the first is that there is a G114R variant in a CALM3--
A. Yep.

Q. --a gene in an individual that we were discussing in the UK Biobank. Now

you have not applied the criteria, the benign supporting criteria to that fact. On the other hand, you have applied, and this is I think the strongest criteria you've applied in support of pathogenicity, in relation to the CALM3-G114W variant--
A. Yes.

Q. --that that's a known pathogenic variant?
A. Yeah, likely pathogenic, yep.

Q. You've applied that at a level of moderate evidence?
A. Yes.

Q. Given, as I take it, you don't agree with Professor MacRae on that, that what he says--
A. I can't remember what he says about it, but if he says it's in a different gene and we can't - we can't--

Q. He doesn't comment on the criteria but given what you've just told us, you--
A. Right. Yeah.

Q. --don't agree with in terms of the - that they're not interchangeable?
A. I mean that's being very stringent. I, yeah, look I disagree with him. I think you can make a case for what he's saying, and he does make a case for it, but I don't agree. I think that the variant in the CALM3 is relevant.

Q. Coming then to red page 56 in your report in Exhibit 7, tab 3, item 2.5, missense variant in a gene that has a low rate of benign missense variation in which missense variants are common mechanisms of disease, we've already discussed that this is a very significant criteria for a number of other experts?
A. Yes.

Q. I think you've already said it would be open to place more weight on this factor--
A. Potentially, yep.

Q. --but you don't place more weight on this factor? In fact I don't need to ask that because you've done it. Coming to criteria 2.6, multiple lines of computational evidence to support a deleterious effect on the gene or gene product. You're referring to things like algorithms--
A. Yeah.

Q. --predictive--
A. Yep.

Q. You've said this type of evidence can be used at a supporting level if multiple tools support pathogenicity?
A. Yep.

Q. Do I take it from that that the guidelines don't permit you to give it anything more than supporting evidence?

A. So, there's a very recent publication that outlines some situations where you potentially could.

Q. Is that a publication you've referred to, or?
A. No, I haven't; it came out after this - I think it came out after this - in fact, I'm - I think it's published in an issue, it was a preprint when I saw it, but I think it's been published.

Q. Is this referring to the Flloyd - I'm going to take you to it and you can tell us and if not, we'll come back to it.
A. Yeah, okay.

Q. In the report that's found at Exhibit 5, tab 7, of Professors Arsov, Cook and Vinuesa, which responds to the further materials of Professors Overgaard and Nyegaard, at red page 183.

WOODS: Sorry, which report is this?

ROY: This is the report from Professors Arsov, Cook and Vinuesa responding to the further materials of Professors Overgaard and Nyegaard dated 23 January 2023, Exhibit 5, tab 7 at red page 183.

Q. On page 196 using the red numbers - in fact, page 194, excuse me, they report the results of deep-mutational scanning.
A. Oh that, no, no.

Q. No, a different paper?
A. This wasn't the one I was talking about, no.

Q. The paper that you were speaking of, would--
A. It provides circumstances in which you could use a higher level of evidence. One of the things about it is that, again, they're quite strict about how you do this; you can't cherry-pick. The problem is, there are lots of these tools and - and there a couple of them for which they find evidence that you could go for a greater strength of - upgraded classification, but you've got to do it from the beginning, because--

Q. What does that mean, "you've got to do it from the beginning"?
A. Well so, so we've queried 23, say, different in silicio tools and often what you get is that a couple of them have got quite strong-looking scores and a couple have got not such strong looking scores and a couple say the thing is benign. So I think there's 70 in our 20 for the G114R, and when you look at individual scores, they'll vary in their strength. So you can't do that and then go "ah ah, that one says it's really strong". You've got - your starting point has to be "I'm going to use the REVEL score and I'm going to apply that and if it says that there's moderate evidence then that's the criteria I'll use". So because I'd classified this variant before that paper came out, I haven't - I haven't modified, I haven't gone back and attempted to redo the exercise. I don't know what would happen if you did, but you can't, you know, you can't go both ways. You've got to start and go forward. This is something different.

Q. This preprint; you had a chance to refer - so the report of Professors Arsov, Cook and Vinuesa refers to a preprint article.
A. Yep.

Q. Which is found at Exhibit 15, tab 17.
A. One of the other reports says that it's been published.

Q. It has since been published.
A. I read it in my--

Q. That was my next question: Have you seen the published version?
A. No, I've only read it in preprint.

Q. Okay, so I'll confine my questions on the basis that you've only read the preprint. You're aware that that predicts a high likelihood of pathogenicity for G114R?
A. Yes.

Q. Do you consider that as reliable further evidence of pathogenicity that you could include in an assessment?
A. No.

Q. Can you explain why not?
A. The assay that Floyd and colleagues refer to is a screen that is multi-modal or something or other, I can't remember the exactly terminology, a screen that was done in yeast and so what we - that's another paper and I can't remember the first author of that paper, but what those - what that group did was that they made essentially every possible missense change in the calmodulin protein and they put it into yeast and they saw what it did to the growth of the yeast and then they rescued the - the - the phenotype by putting in wild-type, and I mean, there are two things about this:

One is that they classify quite a lot of potential variants as being benign, or at least, unlikely to have an impact on the yeast scale, but in fact, the other problem is that it's not a physiological measure. So yeast biologically are quite different, we share things with yeast, a surprising amount, but - maybe not obviously, but it's true; we genetically share quite a lot with yeast - but growth of yeast is such a non-specific phenotype that I find it hard to place any real weight on that.

And then Floyd et al present, at least in the preprint, three cases in which they say we looked to see - essentially, we looked to see whether this, this score could be used to upgrade the classifications.

In practice, none of the classifications were changed by the score, but they also don't present any evidence that says why you could potentially upgrade or downgrade the pathogenicity of a variant, and I think that's quite a big gap. It seems like a bit of a leap of faith to say we've got the score; now we're going to apply it and see if it works without any of the background work you would need to do - you'd need to do a lot of background work to really work out if it's

predictive of anything in a human, as well as the yeast.

Q. So you would disagree with that as a valid approach to clinical decision-making, to have regard to this?
A. Oh yes, I wouldn't do that, yeah.

Q. I think for the record, the paper authored by Weile is the 2017 report of the yeast study, is found at Exhibit 15-173; somebody will tell me if I'm wrong about that. Coming back to your report, Exhibit 7-03 at page 56, including the factors - your summary at the bottom, "2.7 Summary of evidence in favour of the CALM2-G114R variant being pathogenic".
A. Yep.

Q. I just wanted to understand how you apply those. You say that there's one piece of evidence at moderate strength?
A. Yep.

Q. That was PM5 which was the--
A. The missense variant in - another missense variant at the same location.

Q. G114W?
A. Yep.

Q. That gets moderate strength?
A. Yep.

Q. The four at supporting strength, and I know the other ones we've gone to. You say, "As noted above, it is possible that there is additional evidence available that could upgrade PS3 P" - that's the functional assays?
A. Yep.

Q. "To moderate strength". I think there's maybe--
A. It turns out that that's not the case, I think.

Q. You think that's not the case now?
A. Yeah.

Q. Why do you say that it turns out that that's not the case?
A. Because of the evidence of Professors Nyegaard and Toft Overgaard; they didn't present evidence that - I mean, they are the people who would know and they didn't present evidence of that nature.

Q. Yes, so that could only be upgraded if there was - if it was validated to a diagnostic standard or--
A. Yeah or well, no--

Q. Or you had--
A. You wouldn't have to get to a diagnostic standard to get to "moderate" but yeah.

Q. You had say - tested six benign variants, I think you'd estimated it. Should there be a full stop between "Moderate strength" and "Under", just so I understand?
A. Yes, there should.

Q. Okay, so then you say, "Under the ACMG rules for combining evidence, if this were the totality of the evidence", and so I just want to be very clear, when you say this is "the totality of the evidence", you are not including the possible upgrading of the functional assays?
A. Yeah, yeah.

Q. You are or you're not?
A. I'm not including them, yep.

Q. "this would reach the threshold for classification as likely pathogenic". So one moderate and four supporting?
A. Yep.

Q. Then you come to evidence against pathogenicity.
A. Yep.

Q. This is a single criteria at BS2 - this is on red page 57: "Observed in a healthy adult individual for a dominant disorder with full penetrance expected at an early age". Now, there's a number of elements to that.
A. Mm-hmm.

Q. First of all, ordinarily this would be applied as strong evidence?
A. Yeah and look, I mean, clearly others have done that, and I was - in downgrading it, I was trying to address the possibility that you could have non-penetrance. I mean, perhaps that wasn't the right thing to do; perhaps I should have left it at "strong". And to be honest, really what we're capturing here is that issue about assessing a variant in a person without a phenotype. So the criteria give you a way of kind of expressing that and laying it out, but that's the fundamental issue.

Q. The first criteria is observed in a healthy adult individual?
A. Yep.

Q. Does that require positive evidence of health?
A. It depends on the condition.

Q. Okay.
A. Yeah.

Q. So are you looking only for the phenotype? Are they considered "healthy" for the application of this criteria purely on the basis of absence of phenotype?
A. No, I think you'd need more, and we've got more. We've got the cardiac investigations. You've got to work with what you've got, of course, and I really do think that this is - from the point of view of my classification of the variant, this is what it hangs on is whether or not Kathleen Folbigg is affected by a

relevant phenotype.

Q. You said at the beginning of your evidence that there is a question as to whether Kathleen Folbigg has the phenotype.
A. Yep.

Q. On the basis that most of the cardiologists consider she doesn't; Professor Wilde considers it's--
A. Possible.

Q. --possible. Does that--
A. If that's the right interpretation of what Professor Wilde is saying, yeah.

Q. If it was - given there's a question--
A. Yep.

Q. --is it valid to apply the criteria that includes a requirement of being observed in a "healthy adult individual" at all? As opposed to questions of upgrading or downgrading, can you even apply the criteria?
A. So the question - I guess I'm putting more weight on the lack of clinical evidence and the interpretation of the majority of the cardiologists that the investigations are normal. I mean really, either she is affected or she's not and that's not my determination to make. So I'm working on the basis that she's not affected and that she is healthy, based on the fact, particularly in the calmodulin variants, and we're talking about something that potentially might have - in principle, or we're talking about something that might have resulted in two deaths at a very young age, the fact that she's alive and well and has normal - apparently normal cardiac investigations. That's good enough for me for a "healthy individual". But, if - if, you were persuaded that she does have a relevant phenotype then no, you couldn't apply that; you'd have to throw it out the window and indeed, I would classify the variant as "likely pathogenic" without any hesitation if I were confident that she had a phenotype.

Q. If Kathleen Folbigg clearly had a CALM-associated phenotype this would actually not just remove this criteria, but it would be a criteria in favour of pathogenicity?
A. Yeah, in the sense that you'd get to the starting block. So you'd be classifying a variant in a person with a phenotype, which is what we normally do.

Q. Coming back to this idea that we're on the borderline of whether or not anything has been identified, when you say you're taking the approach that she's healthy.
A. Yep.

Q. That is, you're not weighing that there is uncertainty; you are not having regard to the uncertainty and just proceeding on the basis that she was healthy?

JUDICIAL OFFICER

Q. Professor, you do say in your report it's unclear that she's affected.

ROY

Q. Yes.
A. Yeah, there's some - there's some uncertainty raised by Professor Wilde's report.

Q. But what I understood you to just say.
A. Yep.

Q. Is that, for the purpose of applying the guidelines, you've not had regard to the uncertainty; you've instead considered that she was healthy?
A. Yeah, I think that's fair, that's fair to say.

Q. Is that valid? Can you validly apply the guidelines in that way?

JUDICIAL OFFICER

Q. Can I ask that another way? Would you adjust your assessment if you considered it was uncertain? If it was unsure whether she had the phenotype or not?
A. I guess it depends on the degree of the uncertainty and the nature of the basis for that uncertainty. Sorry, that sound wishy-washy.

ROY

Q. Can I put it this way? You downgraded this criteria from strong to moderate on the basis of acknowledging the possibility of variable penetrance, and we'll come back to that.
A. Yep, yep.

Q. Would it be appropriate to further downgrade it on the basis of the uncertainty?
A. So it seems to me like there's not very much uncertainty, and I guess that's probably why I didn't - didn't make that. You've got someone who is alive and well, in her 50s, who has not had, in my opinion, any documented clinical incidents that are relevant to the diagnosis and where there's perhaps a subtle abnormality on an investigation. That doesn't, to me, that's not enough for me to--

Q. When you say there was a subtle abnormality, are you referring only to the CPVT, the ectopic beats?
A. Well, the issues raised in Professor Wilde's report, because he also mentions some possible lengthening of the QT interval post exercise.

Q. I was asking that. That came in after your first report?
A. Yeah it did, yeah.

Q. So that didn't change your view, the possible QT prolongation didn't

change your view?
A. I mean, look. My view is that if she's affected then the variant's likely pathogenic. If she's not affected then for me it's a variant of uncertain significance. My assessment of the evidence from the accumulated cardiologists is that she's not affected, but I'm open to the possibility that I'm wrong. You know, it's hard to know when you've got someone like Calum MacRae saying everything's fine, and someone like Arthur Wilde going well maybe possibly, because they're both really renowned experts in the field and I don't know quite what to do with that.

Q. This criteria's only being applied on the basis of Kathleen Folbigg?
A. Yes.

Q. So, I take it from that that you've had no regard to ambiguity arising out of Laura Folbigg's single lead ECG?
A. No, I have not. No.

Q. Are you aware that there has been some suggestion of potential mild QT prolongation?
A. Yeah, so in fact Jon Skinner was the one who raised that and his conclusion was that the leads essentially are uninterpretable, so on that basis I haven't put any weight on it. It's actually interesting that quite a few people seem to have accepted that the QTc was 380 milliseconds which, if that were the case would be quite strong evidence against Long QT Syndrome, including in the Brohus paper, which is kind of in there without comment.

Q. But in any event you haven't had regard to that?
A. No.

Q. Coming back to the application of this negative criteria, BS2, in your first report.
A. Yep.

Q. Red page 57. Is the second element of BS2 would be that it's in a dominant disorder?
A. Not specifically. It could be in - well hang on. You'd need - so to apply in a recessive disorder you would need them to have a pathogenic variant on the other copy of the gene.

Q. But there's no issue of that here?
A. No.

Q. This is a dominant disorder?
A. Yep.

Q. Lastly, when you say that currently in applying the criteria you're saying that full penetrance is expected at an early age for this variant?
A. Well, that was certainly the basis that we're operating on in terms of the reported cases.

Q. You've already alluded to Professor Schwartz identifying one case?
A. Yep.

Q. That he's identified clearly of a variable penetrance?
A. Incomplete, well, non-penetrance.

Q. Non-penetrance?
A. Yeah.

Q. As you say, variable as to the variant--
A. No, no. No they're both phenotype. It's bad nomenclature. Essentially, non-penetrance is the extreme of variable expression. So, variable expression is how much you're affected, and if you're very, very mildly, eventually you'll get to a point where even with investigations you're not affected at all, that's non-penetrance.

Q. On my rough calculations of Professor Schwartz's updated data, such as he's shared it with us, there are approximately five families that have not been identified in this mosaic on the Registry, assuming this represents only one of those five families, well that's existed, it's a rate of say 10%, I appreciate those are very, very rough numbers, is it still appropriate to apply the criteria that full-penetrance is expected at an early age?
A. Well, that's why I downgraded it.

Q. Well, you didn't--
A. Well, no, I downgraded it because I was trying to keep an open mind about that possibility.

Q. But at that point there was no - when you downgraded it there was no evidence that that had ever occurred?
A. I think we had the Kato paper, and there were people who were affected, but quite minimally. And also I think in the original CPVT paper there were some individuals who were clinically fine. So, you know, I couldn't rule out the possibility.

Q. Clinically fine but on investigation some--
A. Some abnormalities, yeah. Which, you know, you can certainly imagine that there might be people who didn't have any abnormalities.

Q. The fact that the concrete case has been identified out of a relatively small pool--
A. Yep.

Q. Does not change your weighting of that criteria?
A. So, I think you're referring to the second of the three case examples?

Q. Yes, the second of the three, and I'll open it--
A. --that Professor Schwartz, so I guess one of the striking - so there are a couple of things about that. He doesn't tell us what the variant is. So, that makes it a little hard to assess that aspect of it, and there's no information

about what investigations the father may have had, so again, it's hard to independently evaluate that, but the other really striking thing is that we have got a very clear phenotype in the affected infant who had very severe Long QT Syndrome. So, that's where there's the difference. But in answer to your question about the possibility, so if we accept all of that information at face value--

Q. But that's, can I stop you for a second?
A. Yep.

Q. Those factors you would say weigh on how relevant that it is to compare this case to that case, which is purely on the question of whether in assessing this criteria there would be an expectation of full penetrance at an early age?
A. To get to strong evidence yeah, and not that many conditions have full penetrance at an early age.

Q. But does that one case, so as to take it at its highest, that he's described it, would one in ten, say, cases suggest that this criteria can't be met, full penetrance--
A. Sorry, one in ten?

Q. That's my own numbers and I'll withdraw it. That's not helpful.
A. So, I guess one of the things about this gene is that the great majority of people who've been reported, that's still *de novo* for the great majority, so the new cases Professor Schwartz provides information and at the time of the previous Inquiry it was 84% of families had a *de novo* variant, now it's 82%, so it's still the case that the great majority of the time these are lethal conditions or potentially lethal conditions that are mostly inherited *de novo*.

Q. The effect of that being that nothing in that new information changes your view as to what weighting should be given to this criteria?
A. I don't think so.

Q. You don't think so?
A. No, I don't.

Q. You're not expressing--
A. No, it doesn't. I'm not changing my view. Because I'd already kind of incorporated the possibility.

Q. In assessing, again, assessing the same criteria whether you would expect penetrance at a young age, in respect of CALM-G114R, is it relevant to consider the data for other genes that are associated with Long QTS or CPVT, so outside of the calmodulin genes?
A. Potentially, I think so. Yeah. Because CPVT associated with RyR2, and CASQ2, there's an interaction between the two proteins, between calmodulin and RyR2 and so I think the hypothesis is that the reason you get CPVT relates to that in some families with calmodulin variants relates to that interaction. So, potentially, the clinical manifestations of CPVT related to other genes might be also relevant to the calmodulin genes, but I'm not certain about

that. I think there may be room for doubt and I wouldn't claim the expertise to make any kind of authoritative statement about that.

Q. Professor Schwartz, in his addendum, Exhibit 8, tab 4, red page 38-9, he, at the bottom of that page, we'll just give it a moment, there is describing the Moss-Schwartz Registry?
A. Yep.

Q. Which he says contains data for several thousand patients--

JUDICIAL OFFICER: Sorry, what page?

ROY: It's page 38-9.

JUDICIAL OFFICER: Yep.

ROY

Q. He's referring there and it's not important to read it specifically if I can't find the precise quote but it describes many families in which there has been an early sudden death or cardiac arrest with an alive parent carrying the same mutation?
A. Mm.

Q. This is of the Long QTS Registry. And it makes the general observation that the fact that clinical severity can dramatically worsen in a new generation is standard knowledge, and I accept I don't have specific numbers for you, if that be true, that there are many families in that Registry, the Long QTS Registry?
A. Yeah, so the other genes that are associated with Long QT Syndrome behave rather differently and penetrance is definitely incomplete for several of those and expression is very variable, so you get people who live their entire lives without a problem and potentially with only a mild abnormal or even a normal ECG and other people in the same pedigree with the same variant who have a lethal event at a relatively early age, so that definitely does occur. That degree of variability. One of the striking things about the calmodulin genes is how little variability there is compared with the other Long QT genes.

Q. Is that comparison, you compared that you don't see that in calmodulin genes. Is that a product of how young - how early the research is into calmodulin and how constrained the variant is - the gene is?
A. I don't think so. Clearly, any time - so any condition that's described tends to be described in more severely affected people first. That's just a general rule in genetics because the people who are more severely affected are more likely to get investigated, and so we see a widening of the spectrum of severity essentially with everything. That's a general rule. There are doubtless exceptions but it's a thing we see a lot. But the people who were first investigated with Long QT weren't like the people in the calmodulin families. If you go right from the beginning one of the difficulties in identifying the genes which used to be a process of mapping through families was working out who

was likely to be gene carrier and who wasn't, because there were people who had mild or no features. So, I think this - think the calmodulin's are fundamentally different from the other Long QT genes in that regard.

Q. You would say following from that that it's not valid to look to the experience of Long QT in that respect in terms of applying this criteria, BS2?
A. I think the general point Professor Schwartz makes is right. We do expect that there will be a broadening of the phenotypic spectrum. So, I don't disagree with that. I mean, the other thing that we may find is that there are benign variants we don't know about as well, you know, Professor Schwartz urges humility in terms of our lack of knowledge which is totally fair, but it goes in both directions.

Q. And in any event it doesn't cause you to downgrade the criteria of BS2?
A. No, I don't think so. Because I kind of have already.

Q. I think it's been accepted that there's an accumulated view of cardiologists in this Inquiry but that's based on incomplete information. Sorry. You're aware that a number of experts that have given, cardiologists who have given evidence, and or assisted the Inquiry with reports, have recommended additional testing?
A. Mm-hmm.

Q. Most recently in Ajmaline?
A. Ajmaline.

Q. Ajmaline, thank you?
A. That's the way I pronounce it. It's not a test I have ordered myself.

Q. I will defer to you on the pronunciation. Does the absence of evidence that might otherwise be answered through further investigation affect the weight that you can apply to the presently accumulated cardiologists' view as to Ms Folbigg's phenotype?
A. We're used to dealing with uncertainty in genetics and we of necessity deal with the evidence that we have. It would be nice to have more evidence. It would've been very nice if someone had thought to do ECGs in these two unfortunate children which might have provided us with more information or potentially identified a condition that could've been treated if they indeed did have Long QT Syndrome but we don't have that. So, you know, although the counts of perfection is to get as much information as you possibly can, the reality is that we seldom have everything that we might want.

Q. I'd like to come now to your concluding view as to whether or not it is likely that the variant occasioned the sudden deaths of the two infants?
A. Mm-hmm.

Q. Or young children while asleep?
A. Yep.

Q. Sitting here today, everything you know now, having seen the reports of

the other experts, what is your view as to whether or not it is likely that this variant occasioned the sudden death of Sarah and Laura Folbigg?
A. I don't know.

Q. What is your view as to whether or not it is likely that this variant would occasion the sudden death of two infants, not specifically the Folbigg children?
A. So, we've seen there has been evidence presented that if the variant's pathogenic it's likely to be associated with a CPVT phenotype or with a mild Long QT. That's I think in the Brohus paper, and it's certainly what Professors Toft Overgaard and Nyegaard said. Mild Long QT would not normally be expected to cause two deaths at a very early age. I don't know how much weight you can place on that functional assessment though. I mean they could be wrong in the other direction. They could be under calling the severity of the variant. Look, I think it's possible that it could've caused the deaths. I think it's plausible that it could've caused the deaths. I don't have enough evidence on which I can say I think it's likely that it caused the deaths.

JUDICIAL OFFICER

Q. On the material available to you, can you exclude as a reasonable possibility that the death of these two children resulted from them having the CALM mutation?
A. No.

ROY

Q. In view of that, can I clarify? If we go to page 12 of your first report, which is Exhibit 7, tab 3 at red page 58, you said in the second paragraph on that page, "In order for the variant to be included as a possible cause of the two deaths, the following would have to be true"?
A. Yes.

Q. And the first, "We would have to accept that Kathleen Folbigg does indeed have evidence of CALM disease, in the form of a partially concealed CPVT phenotype". Do I take it from what you've said today in view of--
A. Yeah, you're right, that's overly strong. Yeah, I withdraw that.

JUDICIAL OFFICER: Which part is being withdrawn?

ROY

Q. 3.1.1?
A. So, I - yeah, because I think it is a possible cause and so, you know, we don't have all of those things, not with confidence.

Q. The next criteria, 3.1.2, you say, "We would have to accept either that CPVT was indeed the cause of the two children's deaths"--
A. Yep.

Q. --even though this has never been seen before in these circumstances, or

that both children had severe long QT. Accepting what you've just said about the first criterion--
A. Yeah.

Q. --"whilst their mother had CPVT and no evidence of Long QT, despite having the same genetic variant"?
A. Yeah, I think that's correct, yeah.

Q. Now, considering Long QT Syndrome, the only possible cause of death, you've inferred that from the CALM Registry, calmodulin Registry data? That the only causes of death on that Registry are Long QT Syndrome?
A. Where it's known.

Q. Where it's known?
A. Yep, at that sort of age, yeah.

Q. That's a dataset of well 79 I think at the time that you looked at the data?
A. Yeah.

Q. Is that a large enough dataset to draw a picture about what is reasonably possible as to the phenotype of the variant?
A. Well, as I said, the range of phenotypes always expands, so you can't say from those sort of numbers what could possibly happen, you can only say what's been observed.

Q. So would you withdraw that as well in terms of that being as central to concluding possibility--
A. I'd maybe qualify it, so based on what we currently know.

Q. You're aware of the additional work of Professors Toft Overgaard and Nyegaard showing a possible effect on the sodium channel?
A. Yes.

Q. Which was also consistent with the location of the variant at the binding site for the sodium channel?
A. Mm.

Q. You're aware - and I clarify, no expert is suggesting that that proves Brugada Syndrome would be associated, but you're aware that Brugada Syndrome is otherwise associated with variants that disrupt the sodium channel?
A. Yes.

Q. Are you also aware of an article that was published just a few weeks ago on 29 January 2023, which is at Exhibit 15-263? There is no criticism of not having read this paper, it only came to our attention, but if we could pull that up, "Arrhythmia-associated Calmodulin Variants Interact with KCNQ1 to Confer Aberrant Membrane Trafficking and Function", the lead author is Po Wei Kang of Washington University. It is at red page 3687.
A. KCNQ1 is a potassium channel.

Q. Potassium channel, quite?
A. Yeah.

Q. I will ask you to turn up red page 3688 behind tab 263. In the method and results there, first of all, are you aware of this article?
A. No - you showed it to me this morning I think but not before that.

Q. It's noted in the method and results the second sentence at line 12 on the page:

> "We identify one variant (G114W) that exhibits severely weakened binding to KCNQ1 but find that most other CaM variants interact with similar binding affinity to KCNQ1 when compared to CaM wild-type over physiological Ca^{2+} ranges."

I'm not going to pretend to be able to perfectly decode that.
A. Right.

Q. If we assume for the moment that that report's on a functional assay not the same, of a similar kind, to those that had been conducted by Professor Toft Overgaard in respect of sodium, but that this one is in respect of potassium?
A. A potassium channel, yes.

Q. That there has been demonstrated effect on G114W?
A. Mm-hmm.

Q. Just take that from me for now?
A. Yep.

Q. For the sake of assessing whether or not it is possible that this variant was responsible for the death of the girls, having regard to that as well as the Toft Overgaard assay in relation to the sodium channel, would it be reasonable to include a third category of possible cause of death - being an arrhythmic syndrome not previously associated with CALM?
A. I think the evidence of Professor Toft Overgaard is much more relevant. Because if there is an impact on KCNQ1, then that might speak to the mechanism for a Long QT Syndrome because KCNQ1 is associated with Long QT Syndrome, but I don't think it tells us anything new about possible mechanisms. Whereas the potential interaction with a sodium channel, the only - in fact so at the moment, to go back a step with Brugada Syndrome, there's a zoo of genes that have been accused of being Brugada Syndrome genes and really only one of which there is solid evidence, SCN5A, that's the sodium channel I think you're referring to. So, interaction with SCN5A, if that were a confirmed finding, might represent a mechanism by which the particular variant exerts its effects. This might also be a mechanism by which the variant asserts its effects, but that wouldn't be relevant to a condition that's different to what we really know about. We already know about Long QT Syndrome in calmodulin disease.

Q. I am interrupting your answer about Brugada or another arrhythmic

syndrome, but flowing from what you've just said there about KCNQ1 being connected to Long QT, again assuming, I'm asking you to assume on the basis of the paper you haven't read--
A. Yes.

Q. --assuming that there is some support for a demonstrated functional effect on G114W, on that channel, in contradistinction to other CALM variants at other locations, would that tend to reopen the possibility of Long QT as the dominant phenotype, or as a likely phenotype as against CPVT, in this case?
A. Perhaps in theory. It seems like a bit of a stretch, but I mean the most powerful thing is the phenotype that was actually observed, which Professor Schwartz tells us has now been reassessed as being a CPVT. So, that I think overrides the central functional study report here.

Q. Coming back to the work of Professors Toft Overgaard and Nyegaard which you said is more relevant, the way that Professor Watkins put it in Exhibit 14, tab 10, red page 322 in the third black dot point, I might start reading, "Because the calmodulin proteins modulate the function of several key cardiac ion channels", he refers there to the L-type calcium channel, the ryanodine 2 protein and now the sodium channel encoded by SCN5A?
A. Mm-hmm.

Q.

> "and because the calmodulinopathies are newly reported and our understanding is still evolving, I believe the chance that a pathogenic variant could have a phenotype different from the conventional one associated with the well characterised channelopathies is high."

A. Right.

Q. Do you agree with that assessment?
A. So, if I understand correctly, what he's saying is it's quite likely that we don't know everything, and I definitely agree with that.

Q. But it doesn't change your assessment in this case?
A. No.

Q. My last question, Professor, is, putting calmodulin to one side, have you come across families in your clinical practice that have more than one significant genetic condition in the same family?
A. Yes.

Q. How often does that happen?
A. I personally have only seen two or three that I can think of, but it's a well-recognised phenomenon and there are some figures - I was looking at it the other day, in diagnostic exome sequencing in children with intellectual disabilities, something like 1 to 2% of the time you find two different things. That may be an overstatement but it's not a long way out if that's wrong, and

we've certainly seen double diagnoses in the diagnostic service several times.

Q. I lied to you, it's not my last question. Can you tell us about the concept of segregation in genetics?
A. Yeah. So, we talked about family history at the beginning and one of the things, say you've got a family - with a grandfather, his two children, their four children, and half of them have got some clinical condition in an autosomal-dominant fashion, so it's tracking through the family, and you look at the pedigree and you say that looks like an autosomal-dominant condition. If we're segregating a variant in the family, we're testing as many people as we can - certainly affected, sometimes affected and unaffected - I can go into why we might not bother with the unaffecteds - to see whether the variant tracks with the phenotype. So the question you're asking is, does everybody who's got this condition in this family have this particular variant?

Q. Mm-hmm?
A. If it does then you say that the variant segregates with the family - with the phenotype; if it doesn't then you say you've got non-segregation.

Q. What is the significance of non-segregation?
A. If a variant doesn't segregate with a phenotype and that phenotype is rare and distinctive, it makes it quite unlikely, or very unlikely in most situations, that that variant is responsible for the phenotype.

Q. Is that - from everything you've said, I've not seen you weigh that in the application of the guidelines in this case; does it not come into the guidelines?
A. You need multiple affected people before you can consider segregation evidence and we haven't got any definitively affected people.

Q. Obviously in this case, because segregation - the fact that the boys share the phenotype of sudden unexplained death.
A. Right.

Q. While not being carriers--
A. Yeah, I see your point. I mean, you could potentially say well, the variant doesn't - if the phenotype is just that someone died then you can say the variant doesn't segregate, but just having died is not a strong phenotype from a diagnostic point of view because there are lots of reasons why people, even very young children, can die. So it is not enough of a phenotype for me to apply segregation evidence. It's entirely possible that you could have two or even three different causes of death among four children.

Q. To the extent that other experts in this Inquiry and specifically, Professor MacRae, apply segregation in this case, you disagree with that approach?
A. Yeah, I do disagree, mmm.

Q. How, if at all, have you otherwise taken into account the fact of the boys' deaths in forming your opinions in this Inquiry?
A. Largely I haven't. Largely I've just focused on the variant, to be honest. Let's go back to your question about the family coming to see me and they're

asking about testing in a pregnancy. If the history was of two children who had died and they both had the variant, the way I thought about it would be different from if they then had two more without the variant who also died. It would weaken my confidence in the likelihood that the variant was the cause - unless there were very clear causes for those other two deaths. You know, if you had a very clear explanation that was not the variant then I wouldn't take it into account.

Q. So it would weaken it, but it wouldn't eliminate your concern that the variant was the cause?
A. No, because we do see multiple, rare things happening in families.

NO EXAMINATION BY DR WOODS, MR JORDAN, MS HORVATH, MS LOVE, MR HASTINGS AND DR WATERHOUSE

<THE WITNESS WITHDREW

ROY: Your Honour, we don't have another witness until Professor Wilde at 5pm.

LUNCHEON ADJOURNMENT

JUDICIAL OFFICER: Yes, Ms Callan.

CALLAN: Thank you. I call Professor Arthur Wilde.

AUDIO VISUAL LINK TO AMSTERDAM COMMENCED AT 5.02PM

ARTHUR WILDE

JUDICIAL OFFICER

Q. Good morning, Professor, I think it is?
A. Yes. I'm here.
Q. Would you take an affirmation?
A. So, my name is Arthur Wilde, I'm a Cardiologist in Amsterdam in the Netherlands, and I have seen all the documentary in this case and I've studied it extensively. I wrote a few letters on it as you may have seen.

<ARTHUR WILDE, AFFIRMED(5.03PM)

<EXAMINATION BY MS CALLAN

Q. Professor, you've already indicated your name. You've provided the Inquiry with a one page curriculum vitae, for the record, your Honour, it's at tab 9-03, red page 52. Could I have that shown to the witness? Just confirming you can see the document that's being displayed on the screen, Professor, in a moment?
A. Yeah, okay. Yes, this is what I sent on your request.

Q. Okay. By reference to this curriculum vitae I just wanted to focus on your particular areas of expertise. Against the background, as I read it, of being a medical doctor, you're a Professor of Heart Failure and Arrhythmia and also of Cardiology, is that the case?
A. Yeah, I'm a Professor in Cardiology. It started in heart failure and arrhythmia in particular but it became cardiology in general.

Q. Okay. Is it the case you're presently Head of the Department of Clinical and Experimental Cardiology at the Amsterdam University Medical Centre?
A. Yes, I am.

Q. Is it correct that you both see patients but also supervise laboratory experiments?
A. I see patients for the main part. I don't supervise laboratory experiments anymore but my immediate colleagues do. We are in a research group with laboratory physicians and with clinical physicians.

Q. Have you previously supervised laboratory experiments?
A. Yeah, but that's 25 years ago. So, that's really a long time ago.

Q. Okay. You are the lead author of an Expert Consensus Statement on the state of genetic testing for cardiac disease which was published last year?
A. Yes, I am.

Q. I'd like to come back to that Statement in a few minutes, but in general terms it appears to address the principles of best practice in relation to genetic testing for cardiac disease?
A. Yes, it's based on the experiments that were available up to I'd say late 2021. That is all in that document.

Q. That includes Long QT Syndrome?
A. It does, yes.

Q. Catecholaminergic Polymorphic Ventricular Tachycardia or CPVT?
A. It does, yes.

Q. Brugada Syndrome?
A. Yes.

Q. And about six other disorders?
A. Yes.

Q. Would it be fair to describe you as having significant expertise with respect to diagnosing genetic cardiomyopathies?
A. I think so. I've been in this field since 1995 and that was the onset of the field so I think I'm quite experienced, yes.

Q. Do you consider you have expertise in considering the relevance of experimental laboratory results for the purpose of clinical diagnoses?
A. I think I do to some extent, not all diseases are based on the same path or physiological mechanisms but the majority of them I do understand well based on my lab experience in my early career.

Q. Is it the case, Professor, you have a particular interest and expertise in the genetic causes of sudden cardiac death?
A. Yes.

Q. Within that interest and expertise there's one subset and expertise in relation to CPVT?
A. Yes. It is, yes.

Q. I understand that you, together with a group of colleagues run an international CPVT Registry?
A. Yes, we do. That was started six years ago. Six, seven years ago.

Q. Are you able to tell the Inquiry how many patients are presently listed on that Registry?
A. It's close to 1,500.

Q. Are they patients from around the world?
A. They are patients from around the world, yes.

Q. In brief terms, how does the Registry receive data in relation to these patients?
A. So, we have a set limit that the centre should have at least ten patients in order to submit data to the Registry because we want a centre with some expertise at least and if you have only one patient you cannot say there is a lot of expertise, but ten in a rare disease, ten is something. Then we have a special case record form which is sent around and physicians are asked to fill in the data they have available on these cases, and that is what is sent to us and we fill that in in the general database.

Q. Having regard to the nature of the Registry, is it appropriate or are steps taken to verify the data as it's provided to you in relation to the patients?
A. We have done so in the initial phases, and certainly in the centres that have large submissions, so there are a few centres: ours, like the group in the Mayo Clinic in the US, the group in Paris that have submitted significant numbers, and one of our PhD students has been there and checked for the consistency of the data and whether the data are all right. At the other end we

have to admit that centres from Japan for example, we have not been there ourselves, so we rely on the contributions of our colleagues.

Q. As I understand it, a method to seek to ensure the integrity of the data is what you've described in terms of a centre that has at least ten cases so that it's experienced physicians who you're dealing with?
A. Yeah, and it - these are general also physicians that have interest in this topic, so we assume that they are as us convinced that we all rely on these data and as the Registry is going to be the basis and is the basis for most of the recommendations in the guidelines, they know how important it is that the data are reliable.

Q. Yes. Now, can I just check, in terms of the CPVT patients in the Registry, does that span all genetic variants with the CPVT phenotype?
A. Yeah, in principle it does, but the vast majority is related to the most common cause of CPVT which is based on the ryanodine receptor gene, and that is, I would - I think it's over 90%. Maybe even over 95%.

Q. The Inquiry has already heard some evidence about the International Calmodulinopathy Registry, can I ask you this, does the CPVT Registry include the CALM variant cases with a CPVT phenotype?
A. I must admit that I am not sure, I don't think so because in the CALM Registry many of the variants have the potential of an overlap phenotype, so evidence of another phenotype, and that is what we tried to exclude in the CPVT database because we wanted to be a pure CPVT database and not one that is overlapping with Long QT, so I don't think these variants are in there.

Q. You're a member of the group that maintains the Calmodulin Registry, is that right?
A. Yeah, in the sense that we contribute data to that Registry, of our patients, yes.

Q. I see. Do you get access to the Registry data, that is the Calmodulinopathy Registry data, other than when it is detailed in a published report?
A. I think if we would ask for it, we would get it, but we haven't asked for it in this particular case.

Q. Do you have any particular expertise in relation to calmodulinopathies?
A. Not that - not more than the patients that I see with this rare syndrome.

Q. Do you see patients with that syndrome?
A. Yeah, we have I think about ten of the inclusions in the Registry of the Pavia group or the Milano group is from our site, and particularly we submitted the family with Idiopathic VF which is one of these rare conditions that is associated with calmodulin variant.

Q. Can I ask, ten patients you say you contributed to the Calmodulinopathy Registry, one was the Idiopathic Ventricular Fibrillation, do any have--
A. Yeah, this is a family. This is a family of - yeah, yeah.

Q. Sorry, one family, are the others in terms of phenotype, do they - mixed or CPVT?
A. I think it's a Long QT in particular, and ten may be too much, it may be six or seven, I don't think I can recall ten now.

Q. Thank you. You prepared three reports I the form of letters for this Inquiry, and as you recognised at the outset of your evidence - your Honour, for the record the first report from Professor Wilde is dated 24 October 2022, it appears in Exhibit 9, tab 24, the second report is dated 9 November 2022, it's at tab 9-35, and a third is dated 24 January 2023 - this isn't right, I'm so sorry, your Honour.

JUDICIAL OFFICER: The first one's tab 9-02 in my folder.

CALLAN: I apologise to the Court, I will just focus on the documents.

JUDICIAL OFFICER: 9-02, 9-03.

CALLAN: Your Honour, it's page numbers that I was skipping to. There's tab 9-01, p 24 is the first report. Then, your Honour, tab 9-02, p 35 is the second report.

JUDICIAL OFFICER: That's the report of 9 November 2022, yes.

CALLAN: Correct. The third report dated 24 January 2023 is at tab 9-03 which commences at red p 46.

JUDICIAL OFFICER: Yes.

CALLAN

Q. Professor Wilde, can I give you a sense of my questions today? I propose to focus on four key opinions expressed in your reports to this Inquiry. The first is the view you've expressed that the CALM-G114R variant is pathogenic or at least likely pathogenic, and I'd like to - come to your reasons for that. Second, the view you've expressed that there are quite strong arguments to favour CPVT as the most likely phenotype associated with this variant, I'd like to ask you about your reasons for that conclusion. Third, the view you've expressed that CPVT is not a very likely candidate for causing sudden cardiac death at a young age or during sleep. And finally, I would like to address how these views give rise to what you describe as a mismatch between the G114R functional data, and the presumed phenotype of Sarah and Laura Folbigg being sudden cardiac death while asleep in early childhood. Can I begin in relation to calmodulinopathy? Would it be correct to describe calmodulin as one of the most highly conserved genes?
A. I think that is a given thing, yes. I'm not - I'm sorry. I'm not 100% sure about the details after reading several of the reports that were added quite lately. There may be a difference between the different calmodulin. From Dr Calum MacRae, I understand, for example, that Calmodulin-2 is less conserved than 1 and 3. But that is not my expertise. In general, I think it's

fair to say that this are very conservative genes, yes.

Q. In a report to this Inquiry from Professor Schwartz - and, your Honour, his report of 8 November 2022, tab 8-04, page 385. Professor Wilde, you may recall seeing this. He says that calmodulinopathies are fundamentally different from single ion channel mutation-induced LQTS or CPVT. He explains the basis for him saying that: "Because the calmodulin protein is used as a calcium centre by many of these ion channels. Thus, by definition, calmodulin mutation carriers have a very high likelihood of presenting mixed or more complex phenotypes than single ion channel mutation carriers." Do you agree?
A. Yeah, I think in general I agree with that because it isn't - it is not a direct - if you have an ion channel mutation, then the effect of that mutation is related to the action of that particular ion channel. If you - if you have a variant in calmodulin that affects the calcium centres of many other ion channels, then almost by definition, you will have a more diverse phenotype, yes. Because you affect more ion channels.

Q. Can I turn to your assessment of the pathogenicity of this variant. When you used the word "pathogenic", are you invoking any particular definition, such as from the ACMG guidelines?
A. Yeah, so the - I think if you're strictly follow the ACMG guidelines, as Dr Skinner and Dr Cook does, I think you won't end up with a - with the - with - that this variant is pathogenic. But I have some agreement with others, that because it's a calmodulin and the functional data are there, it's a single variant that has not been described in any other family. That is one of the arguments for the ACMG criteria, that it cannot be called as pathogenic or likely pathogenic. But in a case like this with functional data, I tend to agree that it is at least likely pathogenic, so that would be class 4. I don't think you can say it's pathogenic, but likely pathogenic is a fair choice for me for this particular variant, yes.

Q. As I understand your answer to that question, you have not strictly used the guidelines to arrive at your conclusion. Is that the case?
A. Yeah, but you don't use strictly the ACMG criteria. I don't think that this can be called as guidelines because not everybody agrees with it. But I would say that it's fair to say. So, it's - and likely pathogenic means there is a 90% chance that it is pathogenic.

JUDICIAL OFFICER

Q. Professor, you don't use the criteria because it's inappropriate for a single variant. Is that the number?
A. That is. That is by definition. According to the ACMG criteria, you need repeated findings for particular variants in order to be definite on the status of pathogenicity. If you have a single variant in a single case, you can never make - call that a pathogenic variant. By definition, not.

CALLAN

Q. Do those represent the various criteria that cause you to arrive at that conclusion, that it's pathogenic - sorry, likely pathogenic?
A. Yeah. Yes.

Q. Your first report addresses what appears in, what I'll describe as the "Brohus article", which set out the analysis of data from functional assays which have been conducted on the G114R variant. And you expressed the view in your first report that the most likely phenotype caused by the variant G114R would be CPVT. Since providing that first report, more evidence has been provided to this Inquiry, including, you would have seen, data or information in respect of additional functional assays which have been performed, and are described in a report from Professor Toft Overgaard of November 2022, and you addressed that in your third report to this Inquiry. And there are also the updates to the information which appears in the Calmodulinopathy Registry as described in the report of Professor Schwartz also of November 2022. Can I just confirm, does that further information or any other information you've received to date affect the view you've expressed as to the phenotype you would predict for G114R?
A. Not in particular. The relevance of the latest edition of the - of my Danish colleagues is that they have some evidence that the variant may impact on the function of the sodium channel, and the sodium channel was not yet involved in the whole story. It was the calcium channel for the Long QT Syndrome and ryanodine receptor for the CPVT phenotype. Now, the sodium channel is relevant here because sudden death during sleep at whatever age is related to one of the sodium channel disease which is referred to as Brugada Syndrome. Now, that is the speculation that the Danish colleagues add to the case is that when there is some effect on the sodium channel, you might actually have a phenotype of Brugada Syndrome, and that potentially could then be causal to the deaths of these two children.

The problem at this point is that they - these are very preliminary data. There are no functional data on the sodium channel itself, and they made the comment that it needs - that they need a couple of months before the data would be there.

So, what I did in my letter, I speculated on the three different options when you affect the sodium channel, what could happen. You can increase the function of the sodium channel, you can decrease the function of the sodium channel, or you can not affect it at all, and I speculated on that. And Dr Schwartz does not allow me to speculate in a case like this, but I think it's fair to, if you speculate on all three options, that is the only way to proceed, and otherwise, my statement would have been, "I cannot say anything because the data are not there yet."

Now, in case you reduce the sodium channel function - that is the relevant part here - then you may lead to a phenotype that is referred to as Brugada Syndrome, and Brugada Syndrome is a disease that, as I mentioned, can lead to sudden death at whatever age, and in particular, during sleep.

The evidence for Brugada Syndrome is not in the files. There are no

ECGs. You need ECGs with the leads placed on the chest, and that is not in the files for the two children. There is one lead for - from Laura, I believe, but that is not an appropriate lead for making this diagnosis. There is a full ECG of Ms Folbigg herself, and that doesn't show any evidence of Brugada Syndrome. But there is a way that you can test whether she is sensitive to Brugada Syndrome, and that is a drug test that I mentioned in my third report.

Q. Yes.
A. And the sensitivity of that test is so, in my belief, that if there's no response to the drug, then Brugada Syndrome is very unlikely, if not it's not there. If there is - if she does have a phenotype of Brugada Syndrome by that drug challenge test, then the issue is that the specificity of the test to show that she has it is not 100%. It's probably much lower than that, but then at least I would believe that there is a fair chance that that proposed effect on a sodium channel is there.

And I think the other two options, the variant can also lead to an increase in sodium channel function that would lead to an LQT phenotype, and these are usually very severe Long QT phenotypes, and that is not what we see neither in the mother, nor in Laura on that one ECG strip where you can judge the QT interval.

Q. That being the case and recognising, as you've said that whilst you were undertaking an exercise in speculation, you were seeking to provide a fulsome answer to the question as to--

JUDICIAL OFFICER: I prefer to call it exploring possibilities.

CALLAN

Q. Sorry. You were exploring the possibilities that--
A. Yeah, but that's what I'm doing.

JUDICIAL OFFICER: Yeah, exactly.

CALLAN

Q. Does that take you back to the functional assays that were reported in the Brohus article in terms of what they indicate as to the variant in terms of predicting a phenotype?
A. Yeah, so if that - so, that is not on the sodium channel. That was the last information. This is on the calcium channel and on the ryanodine receptor, and I think the overall evidence in that paper, and that's also what the authors themselves acknowledged, that it's more conceivable with a CPVT phenotype then it is with a Long QT phenotype.

There are some differences between the other two variants that they compare with. The change that leads to the Long QT phenotype is a little less expressed in the particular Folbigg variant compared to the two others, and that leads to the conclusion and also the authors conclude that the CPVT is

the most likely phenotype.

Also, if you judge the clinical evidence in the mother, that everything that she shows, if she shows anything, it's very discrete all, but it's most - if it's there, it is compatible with the CPVT phenotype and not with the Long QT phenotype.

Q. Professor Wilde, earlier this week, the Inquiry had drawn to its attention a preprint article which addresses arrhythmia associated CALM variants' interaction with KCNQ1. The article reported on certain functional assays, and the lead author of the article was Po Wei Kang, K-A-N-G. Amongst other things the article states, "We identify one variant (G114W) that exhibits severely weakened binding to KNCQ1, but find that most other CALM variants interact with similar binding affinity to KCNQ1 when compared to calmodulin wild-type over physiological calcium ranges." First, is that an article that you have seen?
A. I think that was the article that was just sent to me this morning when I opened my computer it was there and I haven't read it, not in detail.

Q. Based on what I just described as to one of the things identified in the article, in terms of the variant G114W, exhibiting a severely weakened binding to KNCQ1, does that bear on your view as to the predicted phenotype for the variant?
A. It could, because KCNQ1 is one of the main determinants of the length of the QT interval to say it in simple terms. It impacts on cardio-collector-physiology directly so it could impact on the QT interval directly but whether it lengthens or shortens the QT interval, I have to read the article.

Q. Can I trouble you to do so after you've finished your evidence today at a convenient time?
A. Yes.

Q. And advise the--
A. I will.

Q. Could you advise the Inquiry if having read that article that affects in any way the views that you've expressed?
A. I will, yes.

JUDICIAL OFFICER: Thank you.

CALLAN

Q. Thank you. Can I turn to the phenotypes in the Folbigg variant carriers? Beginning with Sarah and Laura Folbigg, as you've already averted to, there's very limited information about the cardiac position of the two girls. Indeed, in relation to Sarah there is no ECG, and in respect of Laura, there are two ECGs. The first was a single ECG lead, and you refer to that in your first report. Can you identify what are the limitations of it only being a single lead?
A. Yeah, so the single lead, this means mostly, most of the time, and I recall

also in this case I believe it is made when you do an epilepsy study, so a brain electrical activity study. You most of the time have a single ECG leads, and it's not always determined well how the lead is made, what position the electrodes are which is important for the interpretation, and it's - the other thing of the problem of this particular registration is that's quite bumpy so there are quite some artefacts on it and that hampers the exact interpretability of it. But a few things you can still see. On the ECG what you read is the electrical activity of the heart so the heart has the electrical impulse travels with a certain speed through the heart and that is on the ECG read as the intervals between the different parts of the signal. That determines the conduction properties of the heart and on that particular lead you can well establish that conduction in the heart is normal. The conduction between the atrium and the ventricle is normal, the conduction in the atrium itself, and the conduction in the ventricle itself, it's all normal. The problem is the determination of the recovery time of the ventricles which is the QT interval, and that is of course the essential part of this ECG. But I think it's still good enough to say that the QT interval is not very abnormal. There can be some lengthening but it's not - it's not the length that you would expect in a child that just had a serious event from a very long QT interval. A QT interval is a determinant of the chance of having trouble from it, if it's very long the chance that arrhythmias occur is much higher than when it's normal or short. In this case it's certainly not very long.

Q. I just--
A. So, I think it's useful - it is reasonable to say that there is not a very pronounced QT prolongation in Laura.

Q. In your first report, and your Honour, this is tab 9-02, red page 26, I just want to make sure that we understand what you say about this ECG correctly. “The QTc interval cannot be judged accurately enough to exclude significant QTc prolongation which would be expected in the setting of CALM associated Long QT Syndrome." That's in the second last paragraph--

JUDICIAL OFFICER: I have it.

CALLAN

Q. Just reconciling that with the evidence you've just given the commission?
A. Yeah, I have to read back the ECG. I may have been a little bit too conservative here.

Q. Shall we have it shown on the screen for you?
A. Yeah.

Q. Your Honour, that's at Exhibit 2-AC. Page 5332.
A. Yes, and the ECG strip is in the middle here, and I don't think it's very long. Not excessively long, I should say. There is - it could be prolonged a little bit. But you have to do - you have to measure exactly and the problem with measuring the QT interval is that you have to measure from the onset of the QRS, that's easy, but the end of the T-wave is not so easy in a trace like this; and that's probably what I meant with writing that, it is - it cannot be

excluded.

Q. While we still have that ECG, in your third report you distinguish the present case from one reported in Professor Schwartz's report which emerges from the new Calmodulin Registry data where the father had no phenotype and the son had severe Long QT Syndrome. You do so on the basis of Laura's ECG not showing excessive QT prolongation. As I understand it, what you - you--
A. Yes.

Q. --were able to do so as you've described because whilst it's not observable, whilst you cannot exclude it you also - it's not showing such excessive QT prolongation as you would expect for Long QT?
A. Yeah, which is the case in the - so Professor Schwartz uses this small family, the father and the son, to show that there can be marked differences between two individuals carrying the same mutation and the one who has the severe phenotype dies of it and he uses that as a template for this particular family, for the Folbigg family. But, in the case of the example that Professor Schwartz uses, there are good registrations of both ECGs, 12 lead registrations, so that make you comfortable that you can measure the QT in full, nicely and correctly, and that is not the case in the Folbigg family.

Q. The other ECG of Laura is a strip which was recorded by paramedics who were attempting to resuscitate her on the day she died. We might get that put on the screen as well so that we can ensure that we follow what you're--
A. Yeah, that would be nice, yes.

Q. --questions, yes. It's page 5351. From the description of events, is it understood or assumed that CPR was being performed at the time the ECG was taken?
A. It could be. So, the signals that you see on the second line at the end, that is probably CPR artefacts. I think that is - it is not sure, because it's not indicated of course but CPR artefacts is always markedly present and I think that it would be the signals on the - so where you read 12:17:41 at the end of that strip, there are a lot of signals, that is probably CPR, and it continues on the next strip on 12:17:50 and then it stops for three seconds and then it continues. The problem here also is that the time calibration, so I think between one, on vertical stripes, that is one second. That is less than what you see on a normal ECG. On a normal ECG it's 25 milliseconds, millimetre per second, and this is half of it or even less. So it's difficult to read that particular ECG.

Q. A paramedic who administered this ECG described it as showing the child in bradycardia?
A. Yeah, that is - then he is referring here or she is referring to the signals that you see in the top strip and 12:17:32. So you have the vertical lines and through the vertical lines you see this signal which is a white QRS complex potentially, and that is what is seen five times. So that could be bradycardia.

Q. Can I ask you, how would you describe bradycardia?

A. Bradycardia is a slow heart rate and the definition of bradycardia is when heart rate goes under 50 beats per minute, then we refer to it as bradycardia. In this case if the vertical lines is one second, you see one signal every two seconds, so that would mean a heart rate of about 30 per minute.

Q. Is it possible to tell the difference between a resuscitation artefacts and the heart pumping a slow rhythm on its own?
A. Yeah, in general that as well possible. The only exception is when the cardiac arrest has lasted a long time and long is defined as let's say above seven minutes, so let's do one when the resuscitation is ongoing or when there is a cardiac arrest, that is already there for ten minutes, then it becomes very difficult to distinguish from what the heart rate is. You usually see - you don't see any rhythm at all. If you do see a bradycardia with 100% confidence, then the heart is not in fibrillation, so the two are exclusive. You cannot have a bradycardia at the same time of ventricular fibrillation. And if these signals represent the bradycardia, then there is no ventricular fibrillation at this point in time. But my problem with the signals is that they are very similar, so what you see on the top line, there are five signals, they are very similar, the line in between is very straight, so I'm not sure it is really connected well in that top strip. So, I cannot exclude that you are looking at an artefact in the - on the top strip and the first part of the second strip, and that is based on the fact that the line in between the signals is so straight. And the signals that you see are very - very similar, and that's what you would not expect. You would expect some changes between the different signals.

Q. Okay.
A. And so, the connection at the halfway, the second part of the strip, the connection could have been restored, and then you don't see the signals that you see at the top anymore, and then the resuscitation artefact become - become visible.

Q. One observation you make about this strip in your first report is that there is no QT prolongation. Would you expect to see prolongation if there are either bradycardia and, or resuscitation efforts?
A. If the signals are good enough to judge, you would - you would be able to see that. And this is the only strip that is available from that resuscitation? Because the QT is very difficult to judge here.

Q. Yes. You also observed the strip is inconclusive as to the presence of Long QT.
A. Yeah. Yeah. I think that's - that's - it can not be a different answer. It is also - if - and it's important to mention - if somebody is resuscitated, what is happening at the same time, usually different medications are given at the same time and they significantly impact on the QT interval. So, any strip, immediately during resuscitation or immediately after, is not very reliable as to the QT interval.

Q. Thank you, I've finished with questions about that strip. I might bring that down off the screen. Thank you, Professor. The only other piece of information I wanted to raise with you in relation to Laura's phenotype comes

from a statement of a witness that's been furnished to this Inquiry.

CALLAN: Your Honour, it's at tab 14-02.

Q. This is, if I could describe it briefly for you, Professor Wilde, the statement of a witness who was caring for Laura, and she was, according to the witness' recollection, not quite 12-months old. She observed Laura was sleepy and laid on a lounge and went to sleep. The witness left the room briefly at a point in time, and when she returned, she says, "I came back to the lounge room and looked at Laura. She did not look right. She was grey in appearance. I poked her to try wake her up. She did not respond. I listened for breath and felt her chest. My mind was questioning whether I should attempt CPR." And she called the ambulance. She says, "I then scooped Laura up and placed her on the floor. In that moment, she took a breath in. I then sat with her to make sure she kept breathing. She kept breathing in shallow breaths. Gradually, she took deeper breaths and opened her eyes." Based on that description, is there anything you can say from your expertise as to what that may or may not indicate in terms of cardiac function and arrhythmia?
A. Yeah, the problem is of course that's a - that's a very incomplete story. But I think you cannot say that it's not - it potentially is compatible with a cardiac arrhythmia, severe cardiac arrhythmia, that spontaneously terminates, because there was no particular action undertaken to - to revert it back. So, an arrhythmia could be like that. The time intervals are important here; how long has she been out; at one point did it start? That's obviously not known because she wasn't there at the onset. But - but it is potentially compatible with an arrhythmia.

Q. Can someone be, as it were, startled out of an arrhythmia and take a breath?
A. Yeah, if - so, if the arrhythmia stops, so if there is a sudden cardiac arrhythmia that leads to unconsciousness, that is - that is a ventricular arrhythmia, if that suddenly stops, then - then the circulation regains and that can start with a new breath. But my personal belief is that the colour of the patient is particularly important. If - during the arrhythmia, you will be grey or white, pale. And if the arrhythmia stops and circulation resumes, there's usually flush which is a very red, red face. But that is not reported here. But that is sound evidence for an arrhythmia as being the cause of that faint, particular faint.

Q. You've used the term "spontaneously terminates", does that mean that it's not through the intervention or interruption by anyone else?
A. Yes. Yeah. Yeah.

JUDICIAL OFFICER

Q. I see here in the statement that Ms Callan's referring to, Professor, the witness describes her as being "grey in appearance".
A. Yeah. Grey appearance is - is maybe the same as pale. I don't know. But grey is a colour that is compatible with a cardiac arrest, I would say.

CALLAN

Q. Professor Wilde, in your first report, you described the children's phenotypes as "sudden death" at ten and 18 months, respectively. You exclude IVF as most unlikely for the girls, and you set out your reasons for that in the report. You say it's harder to distinguish between CPVT and Long QT Syndrome as the likely phenotype, but you ultimately, as I read your report, that first report, expressed the view that you consider Long QT Syndrome the only reasonable explanation for the sudden cardiac death in the two girls. Can I ask you to address why you consider Long QT is the only reasonable explanation compared to CPVT?
A. Yeah, so let me go by these three phenotypes and start with Idiopathic VF. Idiopathic VF is extremely rare, if described ever, in a young child of this age, and I believe the reason for that is for Idiopathic VF - for ventricular fibrillation in general, you need a certain size of the heart, and in very young children, the heart is obviously a smaller size than if they grow. And in the presence of structural abnormalities or in this presence of very remarkable electrical abnormalities that requirement for ventricular fibrillation is no longer there because then the inhomogeneity and electrical parameters or the structural abnormalities enable ventricular fibrillation, even in a small heart.

So, as the heart was structural normal, and they were small by definition because of the age of the children, I consider Idiopathic Ventricular Fibrillation a very unlikely candidate for the death here.

Now, CPVT was, as I already expressed, mainly based on the evidence from the Brohus paper from the functional data, and on the phenotype of the mother, the absence of QT prolongation, and the phenotype of the mother, if anything, is more compatible with CPVT than with Long QT Syndrome.

The other piece of evidence comes from the Calmodulin Registry where the Long QT phenotype is the most lethal one, and the severity of the variants is made very clear from that first report of the Calmodulin Registry because all the variants, I think, are - from the top of my head now, 93% of the variants were *de novo*, which means that the parents doesn't have it, but the child has it, and the child does not reach reproductive age, so the variant cannot be reproduced, and the child dies at - most of the time at early age, or is resuscitated successfully, and the remaining 7%, has this mosaicism, which means that it is derived from one of the parents, but it is not expressed in the heart of the particular parent. So, that means that these variants that lead to Long QT Syndrome are very malignant and are almost not compatible with life, unless somebody is resuscitated successfully.

Now, in the update of the Calmodulin Registry, Dr Schwartz argues that there are now a few more families with reduced penetrance. The - when I made the calculation, there are only three, so the number of *de novo* variants has dropped from 93% to 87%. The remaining 7% of mosaicism is still there, and that adds up. So, there's only 6% chance that the variant in one of these things are associated with Long QT Syndrome has reduced penetrance. So, that is a small percentage, and that is not much different from the first report.

Now, if you then - the three families that are in that second - in that updated Calmodulin Registry, one is from the Kato paper, and I argued in my first report or my second report, I don't recall, that the QT interval there is still prolonged in almost every individual. So, when it comes to penetrance of the QT interval, the penetrance is still very high.

The second family is the family that we just discussed with the father and the son, and the third family, I don't have the details, but it is still very unusual that you have a reduced penetrance or a very marked difference between the phenotype of two different individuals in the family.

And that is what is - if the variant in the Folbigg family leads to Long QT Syndrome, there is marked disagreement between the - what happened to the children and the phenotype of the mother. So, that is why also coming to the conclusion that the most likely explanation is CPVT is because the LQT phenotype, as we know, results from calmodulin variants. It's not seen here.

So, these are my arguments to, and particularly in the initial reports, that there is a mismatch between the functional data. The mismatch is that the functional data seemed to show the CPVT phenotype. That is what you see in the family, so that there is a match there. But the phenotype of the children is so severe and at such a young age, and also under circumstances that are not usually seen with CPVT, that there's no match there. That is the mismatch.

Q. When you say, "circumstances", do you mean whilst asleep?
A. Yeah, that's what I mean, yes.

Q. Sorry to interrupt. To the extent that you refer to the cases which appear on the Calmodulinopathy Register in arriving at your ultimate view, do you consider the data in that Registry, in terms of the number of patients present, permits you to arrive at conclusions - sorry, I'll start again. Do you think it comprises a sufficiently large data set to exclude the possibility of a death by CPVT in these circumstances and at that age?
A. That is an important question because the problem as I mentioned in my first or second report, the problem of registries is always that it starts with the most severely affected individuals, and over time you will see that in registries the number of additional individuals will have a less severe thing of that. That's happened with other diseases, all these rare diseases, and that's what you see what's happening in the Calmodulin Registry, and the second Registry, if you want, is a less - has inclusion of less severely affected patients than the first. It's well conceivable that a third registry would double the number of patients or triple or whatever, would have more inclusions of less severe patients. Whether this is - this is the general principle. Whether this is going to happen with the Calmodulin Registry is not sure. We know calmodulin now for ten years, and within these ten years the number of severe cases in the Registry is still very severe, it is not 93 for Long QT, it's not 93% *de novo* variants, but it's 87, which is still very, very high. So, I doubt that in the case of the Calmodulin Registry if we would have an update of let's say double or triple the amount of patients that it would be very different. I think it will still be the general principle that Long QT related to calmodulin variants will

express as a very severe phenotype, and the majority will remain - will remain *de novo* variants or cases of mosaicism.

Q. The CPVT Registry, as I read your report, maintains that age 3 is the youngest ever recorded age of death. Is that correct?
A. Yeah, that is correct.

Q. Do you rely on that in making the observation you do as to how unusual it would be for death at a younger age?
A. Yeah, I do. I think it's also fair to do because the CPVT Registry already has 1,500 inclusions. It includes all the centre's work as we mentioned in the beginning of this Inquiry. It includes all the major centres in the world with interest in this disease, so also there if the number of patients would be double I doubt the information will be much different.

Q. Do you accept the possibility that CPVT may cause death in children less than three years of age but for instance they might not meet the criteria for inclusion in your Registry because there's insufficient information to confirm cause of death?
A. Yeah. Yeah, definitely. That is because the requirement for inclusion on our Registry is a definite CPVT phenotype, and that - so you need evidence for a phenotype. That doesn't have to come from the individual who died but then at least in the family it should be clear that there is a CPVT phenotype. The presence of a ryanodine variants in itself is not enough because we recently learnt a few years ago that CPVTs related to gain of function, ryanodine variants, but we now also know that there is a ryanodine receptor variant can also lead to loss of function that leads to a different phenotype that is not CPVT, so just the presence of a ryanodine receptor variant is not sufficient to be included in the CPVT Registry.

Q. Thank you. Professor, you point to the circumstances of the girls, that is, being asleep at the time of their death as part of your reason for indicating Long QT Syndrome the more likely explanation. As I read your report you say that CPVT cannot be dismissed because the death occurred while asleep. Is that your view?
A. Yeah, that is my view. That is because as Professor Schwartz also pointed out in one of his reports, sleep is not always a low adrenergic state. During the sleep there are phases what we refer to as REM sleep, R-E-M sleep, and that is with adrenergic excitement so there is an adrenergic surge during sleep, several times during the night, and so you cannot completely exclude an adrenergic trigger because somebody is asleep. If it would be a very frequent thing you would expect cases in the CPVT Registry they died during sleep, and the number is small and the youngest age is three, as you referred to. But there are cases described as I mentioned in the report.

Q. Thank you, Professor. Can I turn to what is known of Kathleen Folbigg's phenotype? You assess her phenotype in your first report at, it's red page 27, your Honour, at page 4 of the document. Still at tab 9-02. Professor, you know Ms Folbigg's history of syncope is more consistent in your view with vasovagal syncope that is not a result of cardiac arrhythmia?

A. Yeah, I think that particularly refers to the syncopal events at later age. So, not the very early ones. But there are a few described at later age and that are more compatible, so if it occurs during pain and things like that, panic attacks, that is more compatible to vasovagal syncope than to a cardiac arrhythmia.

Q. Can you also address the position in terms of recovery time for a cardiac arrhythmia versus a vasovagal syncope?
A. I don't think that's a very strong determinant discriminator between the two. It depends on how long the cardiac arrhythmias lasted. If it's very short and there is a faint and somebody and the arrhythmia stops and somebody recovers immediately, probably, where the vasovagal syncope you can have this phase of lethargy for a while after the faint. It takes a while before people stand up. What quite often happens if somebody tried to stand up, they fall down again in the case of a vasovagal syncope, but it's mainly the triggers around it that discriminate it too, so the typical examples is seeing blood, starting to sweat, things like that, but that is usually vasovagal syncope and not an arrhythmia.

Q. You refer to the potential relevance of earlier events in Ms Folbigg's life in terms of a reported syncope. You note in particular by reference to an event as reported when Ms Folbigg was at a swimming carnival that you say it's particularly important whether the event occurred during or immediately after exercise. Why is that distinction important?
A. Because the events that are relevant for cardiac arrhythmia used to occur during exercise or during the adrenergic trigger. If you stop exercise suddenly, there is the opposite is happening, there is a kind of vagal surge in the body, so the adrenergic stimuli stops and the vagus takes over, and that is a moment that vasovagal syncope is more likely. So, if it happens exactly during the exercise, the arrhythmia is more likely to be the case, if it happens shortly after, vasovagal syncope is more likely.

Q. Is there anything particular to swimming which has relevance in considering the possibility of cardiac arrhythmia?
A. Yeah, there is, because swimming is known as a typical trigger in one of the Long QT subtypes, Long QT subtype 1, LQT1. It's also more seen in CPVT. The problem is because there is some exercise involved, I believe, or I don't think it's known exactly, the issue though to mention is that for Long QT Syndrome, Long QT Syndrome we now recognise as a disease with different phenotypes, so LQT1 is completely different from LQT2 and LQT3, and that may also be the case in CPVT and now what we know for swimming and CPVT is that it is seen in the ryanodine receptor-related patients and you cannot take that argument that it will also be seen in CALM-related patients. But in general swimming is a trigger in CPVT.

Q. The question which arises is whether that conclusion can be drawn in relation to the calmodulin associated phenotypes of CPVT or LQTS?
A. Yeah, we have learned in Long QT Syndrome that what you see in one subtype is not relevant for another subtype, and that can as well be the case in CPVT.

Q. Can I take you to some of the comments you've made about the ECGs for Ms Folbigg, including an exercise ECG? We'll get that turned up, your Honour, Exhibit 2-BH, start at page 7648 and then work our way through. This is the exam summary and then the data sits behind it?
A. Yeah.

Q. The feature that you observe in relation to the exercise ECG is minor QTc prolongation three minutes after exercise?
A. Yeah, so that is the same argument that I just mentioned. We have learnt in the past years that QTc prolongation after exercise is particularly pertinent for the subtype 1, LQT1, and is not seen in LQT3 and it's modestly seen in LQT2. So also here it is important that the difference in Long QT subtypes have different characteristics as to the QT prolongation after exercise. If we now try to speculate, if I am allowed, on the subtype with the calmodulin, we simply have no clue because there are not that many calmodulin-related QT patients who are alive and who are where you can do an exercise test and observe what happens with the QT interval during and after exercise. So also here extrapolating the fact that the QT is prolonged after exercise, you cannot use that argument that that is confidently enough associated with Long QT Syndrome related to calmodulin, because it's there in LQT1.

Q. Can I ask you this, Professor, would it be unusual to see a person with no cardiac associated variants with some QT prolongation after exercise?
A. Yeah, there is some QT prolongation which can be accepted as normal. I think the discrimination is there when it's more than 30 or 50 milliseconds. I have to look it up what's decided on that, but also in normal individuals you do see some QT prolongation.

Q. To the extent you see here in terms of the minor QT--
A. This is - this is - I would - discrete. It is - it is not very excessive and certainly not proving that she has Long QT Syndrome. And I think the relevance in this exercise test is more the occurrence of ectopy.

Q. Yes.
A. In my letter, I wrote that the first ectopic beat appears at heart rate 158. That ectopy comes from an unusual place which is not what you expect in a normal individual, so that could be an argument for CPVT.

Q. Is that in terms of a standalone indication and/or in combination with the slight QT prolongation?
A. Yeah, it is not enough. The number of ectopy, the ectopic burden - that's what we refer it to - is also discrete, so based on the ectopy alone, you cannot make the diagnosis of CPVT. You have some typical - in typical CPVT patients, you have some typical arrhythmias. If you see that, it is more likely that you reach a diagnosis of CPVT than with this minor ectopy. So, that - I counted two extrasystoles and one doublets, which is two extrasystoles after each other. That is not a lot, and just based on that, you cannot make the diagnosis CPVT, but it is compatible with CPVT. The ectopy comes from different sides of the heart, which is also an argument for compatible with CPVT, but it's not more than that.

Q. Your reports don't address a 2003 ECG that we have for Ms Folbigg, which I think was provided to you.
A. Yeah, I've seen it last - just yesterday.

Q. Can I ask that that be turned up? It's Exhibit 14-06, red page 228.
A. Yeah, you have to turn it to the left.

Q. We'll work out how to do that, Professor. Whilst that's happening, can I ask you, is the combination of that slight QT prolongation and the ectopic beats unusual?
A. I think the occurrence of ectopic beats is more unusual than the slight QT prolongation.

Q. Have we turned that the right way, Professor?
A. Yes. Yeah, so what you see here is the - what is a little bit peculiar is the aspect of the ST segments in the - if you go to the left of the screen, the top lead is lead 1, the second lead is lead two, and the third lead is lead three, and the ST segment, which is the recovery time, is a bit bumpy. There is an extra bump on it which is a bit unusual. Now, I suspect this ECG is made in a supine position, in standing position, and I say that because if you compare it with the other ECGs that are available, there are differences in the QS morphology. If somebody lays down, supine position, and somebody stands up, the heart will become in a different position, and the ECG looks different, and this ECG more likely is more compatible with the standing position ECG that we just saw in the exercise that - and the ECGs in supine position that are also available in the files.

And that makes it a little bit more difficult to judge. Heart rate is slow here. Everything is normal. The QT interval is normal. But the only abnormality is the little bump on the T wave. Now, there's also referral to the U wave here, a marked U wave. I would not recall there's a marked U wave. This is a U wave, and everybody has a U wave, but this is not a marked U wave.

Q. That, I think, deals with a matter I wanted to raise with you which is in the Brohus article, it's suggested that this ECG showed nonspecific notched T wave and prominent U wave in the absence of QT prolongation. As I understand you--
A. So, that's essentially what I described. The nonspecific abnormality in the ST segment, that is what I described as the bump. I also mentioned there's no QT prolongation, and the author refers to this as a pronounced U wave. I think there is a U wave, but every - as I mentioned, everybody has a U wave, and this may be a little bit more than expected, but it is not pronounced. We have cardiac abnormalities with a pronounced U wave, and that looks markedly different than this one. And also that, by the way, I think is more suggestive for CPVT than it is for long QT.

Q. I've finished with my questions about that ECG. Professor, the Inquiry has received evidence about concepts including variable expressivity and complete penetrance, modifier genes and environmental triggers to, amongst other

things, explain why Ms Folbigg is alive and the girls are dead. Can I ask you first about variable expressivity and incomplete penetrance? Do you regard those concepts are pertinent to the present case?
A. Well, these are general principles in any inherited disease. So, there are not that many inherited disease that full penetrance. There are a few, but in my field in cardiology, they are rare. So, that means that there might be individuals with the same phenotype who have a different expression of the disease, a different penetrance, and a different expression of disease is more or less along the same lines. It essentially comes down to the fact that individuals with the same genetic variant have different expression of the phenotype or phenotype at all. So, these are general principles of inherited diseases and by definition they can be pertinent to this case as well. The modifier genes are one of the responsible mechanisms for this variable expressivity and reduced penetrance so if you have modified genes that aggravate the phenotype you will have a more severe phenotype. If you have modifier genes that ameliorate the phenotype you have less expressed phenotype.

Q. In your first report you, in addressing variable penetrance and expressivity, you refer to the Kato paper and you identify some points of distinction. Do I understand whilst you're distinguishing the present case from the family reported in the Kato paper, that doesn't indicate that you reject the possibility of incomplete penetrance or variable expressivity in the present case?
A. Yeah, so in the Kato paper, the Kato paper is brought up because there are individuals alive with the CALM variant, and that is what leads them to conclude that there is variable penetrance. But if you look at the phenotype itself in more detail and particularly at the QT prolongation, I think all the individuals in the Kato paper carrying the variant do have some degree of QT prolongation. There are individuals who have more and there are individuals who have less, but all of have a Long QT interval. So, there is no variable penetrance as to the QT interval. There is some because the QT prolongation is not identical on everyone but everyone has a phenotype. That's what I mean that I think this is not a good example to show that some people are completely normal and others are completely abnormal.

Q. Recognising this is not a good example of showing that phenomenon do you have a view as to the position in relation to penetrance of this variant?
A. You mean in the variant in the Kato paper?

Q. No, the variant of CALM-G114R?
A. Yeah. So, I made somewhere the comment that by definition you cannot exclude that the variant, any variant can lead to reduce phenotype because the possibility of modifiers is always there. That also holds for this particular variant. And you can never exclude.

Q. I recognise the points you've already made about the ACMG guidelines. One of the criteria in those guidelines which is BS2 requires full penetrance. Is it capable of use or application in your view in assessing this variant?
A. So, full penetrance is not present here. The ACMG guidelines are meant to

obtain certainty about calling a variant, and in the presence of full penetrance it is much more certain that the variant is causal pathogenic, than in the case of incomplete penetrance. I don't think but I'm not 100% sure - I don't think you need that BS2 in order to come to the conclusion that the variant is pathogenic. But if it is this is the strict guidelines of the ACMG that I already mentioned at the beginning that I am not following 100% the ACMG criteria here because I think there is fair evidence even in the absence of full penetrance that you come to the conclusion that the variant does something and that means that it's not, as I mentioned, likely pathogenic.

Q. You already made reference to modifier genes and the potential they have. There is a report that the Inquiry has received from the beginning of February of this year, by Professors Cook and Vinuesa which identifies upon reanalysis a REM2 variant identified in the four Folbigg children. Can I ask if that's a report you've had a chance to consider?
A. Yeah, not in great detail. That also came rather late. It's rather complex material so but if you allow me that I write that in the same letter that I already promised on that other paper I will do so.

JUDICIAL OFFICER: Yes, thank you, Professor.

CALLAN: Yes, thank you.

Q. It may be it would be of assistance if you could do so by reference to a 2018 article by Professor Schwartz which is referred to in that report, and we'll make sure that copies are available.
A. Yep.

Q. Just one moment, Professor. Can I turn, Professor, to as you've averted to the mismatch that you identified based on the information you have available, which has given rise to your conclusions as to the phenotype you predict for this variant, and the likely candidate in terms of the phenotype for the cause of death of the girls? I approach the position this way. In one of your reports you describe this a case as an increasingly complex problem. What do you mean by increasingly complex?
A. Now the initial Brohus paper focussed on the known phenotypes related to CALM variants which is CPVT and Long QT, and I put aside Idiopathic Ventricular Fibrillation. I think everybody realised that the phenotype do not completely match the early deaths of the two children. Then on further studying the sodium channel, which is another potential candidate for diseases that lead to sudden death at young age, it becomes more complex, and because these data are very incomplete as of yet it is very difficult to add that information to the case. The CALM variants are by definition complex and, as we already mentioned, if they impact on all different ion channels then the issue is complex to sort the different phenotypes, and the phenotypes that are clearly associated is Long QT Syndrome, CPVT, combinations thereof, Idiopathic VF. However, there is another one like Brugada Syndrome, it's at present unknown.

Q. Professor, having regard to all the information and the complexity that

you've referred to, that you have been provided to date, in your view can you exclude the reasonable possibility that the G114R variant was the cause of the two girls' death?
A. Sorry, I missed the last part because - a little bit of noise there.

JUDICIAL OFFICER

Q. Can you exclude, as a reasonably hypothesis, that the girls' death was caused by them having the G114R variant?
A. No, I don't think you can exclude but that is - I think it's the principle of biology, never and always don't exist in biology, so I don't think you can exclude.

Q. I understand that, everything's possible, but could you reasonably exclude it?
A. Yeah, well, in what I wrote I think there is a significant mismatch in what is shown as evidence for a pathogenic variant, particularly to the phenotype and the circumstances and the age of that of the two children. To me that is - well, it is always possible but it is not very likely.

CALLAN

Q. Just two final matters to raise with you, Professor, and thank you for your continuing time and patience with these questions. They arise from the report of Professor MacRae that we received last month and I hope you might have received and had a chance to consider.
A. Yes. I have, yes.

Q. Professor MacRae suggests that the evidence that calmodulin variants can cause disorders besides Long QT Syndrome including CPVT is incomplete. Do you agree with that statement?
A. Yeah, I think that is what we earlier - what we discussed earlier. The Brugada phenotype is an option but it has to be mentioned that at present there is not a single case reported. And even if the evidence that the Danish group is working on, or whether or not they CALM variant modifies the activity of the sodium channel, even if that evidence comes to the table then there is no phenotype as yet that is compatible with Brugada Syndrome. And if that becomes available, then, yeah, then it decreases the likelihood that Brugada Syndrome is an option, but as yet it is not there and a single case showing that it would not be sufficient for the scientific community to accept that CALM variants lead to Brugada Syndrome, as I mentioned in my third contribution.

Q. Can I ask you, as a general proposition does the scientific - from your perspective does the scientific community expect that a calmodulin variant can cause CPVT?
A. Yes. Yep. I think that first paper of Dr Nyegaard is convincingly showing that calmodulin leads to CPVT. There is this - there are some efforts performed in recent years based on stringent algorithms where all the evidence for association of genes with particular arrhythmia syndromes has been looked at again and scrutinised, and for the Long QT Syndrome the

CALM genes remain as sound candidates with definites, and strong reasons to believe that there is an association, and the same hopes for CPVT.

Q. Professor, you may be aware from the background information that you have been provided that within the Folbigg family there were two girls who died, Sarah and Laura, and they carried this calmodulin variant. There were also two boys who died in infancy and they did not carry the variant. Did you in arriving at your conclusions about the G114 variant have any regard to the fact of the boys' death?
A. No, I tried to read the - in the initial phase I tried to read what was available, but the phenotype of the boys is so way outside my comfort zone that I cannot comment on that. They - there is no cardiac disease shown in these two boys that I can comment on, and when it's a neurological phenotype, I have no clue, and no expertise.

Q. Recognising that, Professor, I want it to be clear, when you have assessed the pathogenicity of the G114R variant and expressed the views that you have as to the likelihood that that variant was the cause of death of the two girls, have you, in any way, taken into account the fact of the two boys' death?
A. No. No. What is a bit strange in the whole case, and I think I've mentioned that somewhere, if there is a sudden infant death or a sudden death at young age, with nowadays knowledge, you would - in the next child in the family, you would make an ECG at birth or shortly after birth. If that - and that was knowledge that was generated - I think it was known for many, many years already, also in the early 2000s when these children were born. But that the evidence of that is important became more and more available in the years - in the 1990s and the early 2000s.

Now, so if that would happen one time, you may not think of it, but if it would happen three times that a young child died, then I don't understand why there is not an ECG available in the fourth child immediately after birth or shortly after because it is know that this - that sudden death at young age has an inherited cause, and it was also known in the 90s and in the early 2000s. So, that is the only - that's the only thing that is strange to me, that there was no medical information or done an extensive report on the youngest children, particularly on the youngest two in this case after already two sudden deaths at early age and two other family members.

<EXAMINATION BY DR WOODS

Q. Professor, we've come to be aware of a lot of the complexities of this matter, some of which you've described today. You've read a number of reports from other highly qualified experts, as you yourself are, and we're very grateful for your input. In 2020, you were the co-author of an article called, "50 years of Catecholaminergic Polymorphic Ventricular Tachycardia CPVT, time to explore the dark side of the moon". Do you recall that article?
A. Yeah, that was an article where the initiative was taken by my colleagues in Melbourne. Andreas Pflaumer was the first author, I think.

Q. It was published in - yeah, sorry.

A. Yeah, it was published in the journal at your side of the world, and it was meant to show the unknowns in CPVT, if I may summarise so.

Q. The abstract talks of despite there being significant progress, "There are still multiple uncertainties and gaps in our knowledge. Like the dark side of the moon, we cannot see them directly. Unfortunately, clinicians must make diagnostic and therapeutic decisions without solid evidence." You've been very careful in this case to point out the absence of certain evidence in relation to the two children and Ms Folbigg, all of which we've carefully noted.

Notwithstanding the uncertainties which you've referred to, you said this in your summary, which is at page 30 of Exhibit 9-02, your report of 24/10/22: "The results of finding a rare CALM variant is of potential relevance for the unexplained death of the two affected children, the two youngest, both girls, child 3, Sarah, and 4, Laura. Almost by definition, one cannot exclude a causal role of this pathogenic variant." I end the quote there, but you go on to say, "However, there are arguments, as extensively outlined above that make it hard to understand what the exact phenotype would've been." Now, first of all, when you say almost by definition one cannot exclude a causal role of this pathogenic variant, would you explain why you say that?
A. Yeah, there is - I think it's in line with what I just mentioned, that in biology medicine there is not “always” there's not “never”, it doesn't exist. So, if you find a particular variant that is potentially associated with sudden death, it is impossible to exclude that it isn't or that it is. And that particularly relates to these cases where the phenotype is, as I mentioned in one of my earlier contributions, is what I record what I refer to as a dirty phenotype, so there is no documentation of what happened. There is no ECG, there is no exercise test, there is no echo, there's nothing, and there's only sudden death. Sudden death is the end point of the disease, potentially end point of the disease, but it's not set that the variant that is identified has caused that and that and that is by definition, you cannot make that argument. You can make the argument if you have an extensive family with multiple sudden deaths, all have the same genetic variant, all have the same evidence for the same phenotype and sometimes even you have the documentation of - arrhythmia, then it's 100% sure, but in cases like this, it is impossible to have certainty about the cause. So it's all about the available evidence and the available evidence is quite little in this particular case. It is the one lead ECG strip in the girl, it is the full phenotype in the mother, she has been investigated fully, and that is why I come to this conclusion.

Q. Yes, very well, but in this case there is considerable agreement that the calmodulin gene is intrinsically potentially dangerous, is that not the case?
A. Yes.

JUDICIAL OFFICER

Q. Variations to the--
A. Yeah.

WOODS

Q. The variant, I'm sorry, the variant?
A. Yeah. Yeah, so the gene definitely is, the variant is, I don't think everybody agrees, there are arguments by some, Professor Skinner, Professor Cook, Professor in Boston - who not agreed that this variant can be called the pathogenic variant, but others are more convinced that it is, and my personal opinion is a practical one and I already mentioned my reasons for it. I think this can be referred to as a likely pathogenic variant.

Q. A likely pathogenic variant?
A. Yeah.

Q. I think you said that you didn't follow the ACMG guidelines strictly but you exercised as it were a judgment based on your many years' experience and your expertise, is that right?
A. I value the functional data more than the ACMG criteria.

Q. You've seen the functional data coming from your Danish colleagues, Professor Toft Overgaard and you accept that as being sound and properly performed?
A. Yes.

Q. One of the considerations to which you paid attention was the ages of the children and you said at page 27 of your first report of 24/10/22, "Based on the clinical data reported on CALM-CPVT individuals and families, I tend to conclude that sudden cardiac death during sleep at age ten and 18 months is unlikely to relate to a CPVT phenotype". Now, in saying that, you're drawing particular force from the analogy of your own work with the CPVT Registry, is that right?
A. Yes. Yeah, that's right, that is the reference. If you look for cases with early age and during sleep, that was the other argument, and the reference is the International CPVT Registry where we have not witnessed that before.

Q. Right, but you couldn't rule it out as being possible?
A. Yes. I agree, yeah. You know the three year old which is the youngest in that Registry was added at the point in time and I cannot exclude the possibility that in the future younger cases will be added. And this if I may add, it is the combination of the age and the circumstances that is important also. Because sleep is an uncommon cause, it does happen and there are reports that an individual doesn't wake up, so die during sleep, and because what we already referred to as the - there are moments during the night that there is some adrenergic surge, so sleep, if somebody dies at sleep you cannot exclude CPVT, but it's unlikely, and it's both unlikely to die during sleep and it's unlikely to die at such an early age.

Q. You take on board what Professor Schwartz said about that, that there can be during sleep adrenalin surges which create a different situation?
A. Yeah, that's true and that is there is no doubt about that and we all have the experience of a nightmare and that is by definitely an adrenergic surge, so it can definitely happen, but it's uncommon. If you look at the triggers in CPVT, it is very rare that it occurs during sleep. The vast majority is during exercise

or emotion, or an argument or - and swimming as we mentioned, and occasionally it's during sleep.

JUDICIAL OFFICER

Q. Would it be exceptional that it occurred in the sleep in two children of the age of these two girls?
A. I think it's already rare in one child, so if it occurs in two it is - yeah, I think it's exceptional, yeah.

WOODS

Q. Professor, in your report you indicated a concern about the Brohus article, with particular notice to the title of it. Do you recall making a comment about that? You said that--
A. Yeah, I - there is one of the letters that I wrote that I read, I don't recall the name of the colleague, she argued that the title that the paper in whole has a lot of speculation on it in the direction of that the variant is causal to the disease, and that should not be part of the scientific article and I fully agree with that. Scientific articles should be unbiased, should not make any reference to what you think it is in this particular case, and that is also I do not have the title in front of me, but that is also in this title already it is expressed as there is good evidence for that the variant is causal to the deaths.

JUDICIAL OFFICER: In other words, scientists shouldn't get emotional about these things.

WOODS

Q. The title was "Infanticide vs inherited cardiac arrhythmias". In your report you put an exclamation mark against that. Why did you do that?
A. I should read the title, I'm sorry, can I look in my own files to read the title?

Q. Yes, certainly.

CALLAN: Perhaps in fairness the witness could also be taken to the portion of his third report where the exclamation mark appears, which is red page 46.

WOODS

Q. Yes, at 46, of tab 9-03?
A. Well, so let me - the arguments that was raised by the colleague, I don't recall the name, is that there is too much emotion in the paper and too much direction given that the variant is causal to the death of the children. That is already in the title, and I think that is the reason I put the exclamation mark there.

JUDICIAL OFFICER

Q. It's fairly obvious, doctor, it was that the exclamation mark can be

explained by the next paragraph in the report?
A. Then I should read the next paragraph.

JUDICIAL OFFICER: The next sentence.

WOODS: Yes, your Honour, I understand that.

Q. Consider this question, Professor, it is fairly standard practice, is it not, for articles to be written about particular cases which are interesting in medical circumstances. There might be hundreds of these reports every year, people writing entries about some new disease, Lupus or some other disease which has been manifested in a particular person or family. You accept that, don't you?
A. Yeah, but the - if you study a variant like this, then I don't think - there are many examples in the paper that it's clear that the authors believe that the variant is causal to the death and they cannot be sure about that and they have to - they don't leave the option open that it's not.

Q. Was that your interpretation of the article, that they don't leave the option open?
A. Yeah. Yeah, that is what I conclude from the paper and to the contrary they argue only that this is - that this variant is causal to the disease. It is also what you see happen over the years, over the more recent years, they keep bringing up arguments that - to explain that this is - that these children died from natural deaths and that's not the problem for me, but that should in itself not be the reason to do all this.

Q. If you look at the article, the title of the article, "Infanticide vs inherited cardiac arrhythmias", it's seven or eight lines up from the bottom of the page, you agree with me that it would've been impossible to write about these children without disclosing the fact that there had been a court case and their mother was in custody?

JUDICIAL OFFICER: I reject that question. Dr Woods, I don't know if submissions are going to be made that the persons who wrote this article were emotionally involved in it. If it is, you can make those submissions to me. I can read the article and see if it might be ultimately the title is justified.

WOODS: The point I want to make, your Honour, is that there is an obligation of scientific openness in presenting a paper such as that, and it could hardly have been done if in presenting these children with their conditions, were concealed from the reader. The reader would need to know that there was this other consideration.

JUDICIAL OFFICER: Yes, but the question's not directed to that. The question is directed to the title. Professor Wilde has not criticised an analysis based on the Folbigg deaths. What he's criticised, as a matter of science, is the nature of the title, and he said, and it'll be up to me to decide whether it's right or wrong, the conclusion and the absolute terms that was reached was unjustified. They're matters that I'll deal with in due course.

WOODS: Your Honour, it may be a matter for submissions in due course.

JUDICIAL OFFICER: I think it is.

WOODS: Yes, very well.

JUDICIAL OFFICER: I mean, from my part at the moment, I should say this to you. Presently advised, I think irrespective of a person's emotional commitment one way or the other, if someone had them, each of the scientists who's called and the medical profession have given evidence to the best of their ability, having regard to the views they genuinely held. You can seek to convince me otherwise, but that's the position I'm taking at the moment.

WOODS

Q. Very well.
A. May I just - may I add there are hundreds of papers, thousands of papers where an individual variant has been studied in relation to a sudden death case or to whatever, a phenotype, where there's no reference at all to whatever. Maybe other - these other cases have the same background, but you don't need the background to study the impact of the variant and whether the variant can or cannot cause sudden death. That whole background is really not needed for that.

Q. Very well. In any event, it remains your position, does it not, that, "Almost by definition, one cannot exclude a causal role of this pathogenic variant"; is that correct?
A. Yeah, that is - but that is a general principle of biology, as I mentioned before.

Q. Which you were relating to this particular case; correct?
A. Yeah, also in this case, that is true, yes.

Q. Your--
A. I don't, however, if I - you allow me, I don't, however, think as Dr Schwartz argues that that is - that after that comment, the case is closed. That is not true.

Q. It remains your position that the variant is "likely pathogenic", as you've explained.
A. Yes.

Q. Just a couple of further matters, Professor. You referred to the phenotype for Laura, and you were read the affidavit about the incident on the lounge. Had you had that information before, or was that something that was just produced today to you?
A. Sorry, can you repeat that question?

Q. Yes. Do you recall that you were asked a question about a part of her background, the child's background, in which it was reported that when she

was nearly 12 months old, she'd been asleep on the lounge? The lady who'd been looking after he left the room briefly, returned, and then looked at her and found that she was grey in appearance.
A. So, that is the incident we just discussed, and that was new to me. I hadn't read that before.

Q. But you would say that that would be potentially compatible with cardiac arrhythmia.
A. Yeah, the way it is described, it is - you certainly cannot exclude, yes.

Q. Would that be one consideration why, even though you're not favouring the position that a causal connection was demonstrated, you would think that you couldn't rule it out in this case? Is that one of the factors which you would have in mind in being unable to exclude some possible causal connection?
A. No, I don't think because I was not aware of this single episode, so I did not include it in my considerations. What I did was the circumstances of that during sleep, and when something happened a few months earlier, then you need to know the exact circumstances, whether there was excitement involved in particular. That type of things need to be known in order to be able to include it in your evidence.

Q. Right, but looking at it now, does it seem to you to be something that you would take into account?
A. If it is if the circumstances are known and the circumstances - let's assume that - I'm not allowed to speculate but if it is during excitement, that would fit with the diagnosis CPVT, but if it's during a resting moment, then not, and I don't think we know the exact circumstances, you're right.

JUDICIAL OFFICER: Dr Woods, would it help you if I, along with the other material that Professor Wilde's going to consider we send this affidavit to him to see if it alters his view?

WOODS: That would be helpful.

WITNESS: Yeah, I think so. Yeah.

JUDICIAL OFFICER: Yes, we'll do that. That's probably the best way to deal with it I think.

WOODS: Yes, thank you, your Honour.

Q. The reference to Brugada Syndrome that you made in connection with the report from Professor Toft Overgaard and Nyegaard, I think you said that Brugada Syndrome was the subject of a great deal of attention in recent decades and there'd been a number of attempts to link it with different genes. Is that the case?
A. Yeah, so Brugada Syndrome has been linked associated over the years with over 20 genes. I mentioned before that there was an attempt to, from an international consortium, see whether all these associations were actually reasonable or not, and that has been done for the different arrhythmia

syndromes, for Long QT Syndrome, for CPVT I already mentioned, and it has also been done for Brugada Syndrome. Brugada Syndrome was of particular interest because over 20 genes were associated with Brugada Syndrome and based on these stringent algorithms that were used, the vast majority, actually all but one of the associations were downgraded to limited or even disputed evidence.

The only one that stands out is SCN5A, that is the sodium channel, that is the gene and the ion channel that the Danish colleagues now have under study, but all the other variants are not considered any more to be associated to Brugada Syndrome. And that has left the field so no longer tests for these genes. So if you have a patient, the new patient with Brugada Syndrome, five years ago we would test for 20 plus genes and now we test for only one. What I mentioned here is that if the doctors in Denmark would prove that the CALM variant has impact on the sodium channel activity, that would not automatically lead to include the CALM gene as a causal variant for Brugada Syndrome because it's only and it's one, and variation gene criteria become important because association with genes is something different than association for particular variants, and when there is association with genes you need at least more cases than just one, you need at least more cases than - and in this particular case, in the Folbigg case, there is not even a diagnosis of Brugada Syndrome, so you cannot associate the gene, a variant in the gene with Brugada Syndrome in the absence of a clear diagnosis of Brugada Syndrome and any absence of repeated findings wherever in the world.

Q. There is a view which has been expressed by Professor Schwartz and supported by, I think, Professor Toft Overgaard that the calmodulin gene variants or mutations that we're now considering here are potentially quite different from many of the dismissed Brugada possibilities. Do you consider that the particular potential criticality of calmodulin ion the heart processes makes it a very real possibility that the sodium connection may well produce something?
A. It is potentially considerable, yes, but it starts with the presence of phenotype. If the phenotype is not there, then you cannot link those and there are several examples in the literature where associations have been postulated and where further evidence has shown down that it's simply not true. And this also hopes for rare variants, extremely rare variants.

Q. One of the aspects of the phenotype of Laura was the presence of some degree of myocarditis which was observed by a number of pathologists. When you gave your evidence about this a moment ago, you referred to the unlikelihood of there being fibrillation in a very small heart. Is that the thrust of what you said?
A. Yes, and that is meant in a normal, structurally normal heart.

Q. Structurally normal heart?
A. Very small structurally normal heart, it's I possible to fibrillate if I may say that strongly.

Q. But if there be evidence of myocarditis which has potentially caused some

damage to the heart, is that not capable of being a structural abnormality which could cause fibrillation or arrhythmia?
A. Yeah, it is, it is, and so if the finding of - I am not a pathologist, let me put that disclaimer first, and what I understand from my paediatric cardiology colleagues is that in sudden death at very young age you quite often find abnormalities that resemble myocarditis, but in this case it is sufficient, and myocarditis as the cause of that death, it is extremely rare but it can occur and the presence of myocarditis would be similar to structural heart disease and so would increase the probability of ventricular fibrillation in a small heart.

Q. You deal with this in your report of 9 November I think at page 3?
A. Yeah, I think I mentioned it there. If there is myocarditis and if the myocarditis is of sufficient degree, it definitely will increase the probability of ventricular fibrillation, also in a small heart.

Q. Is that by the process of the myocarditis causing a roughening or ripples or ridges?
A. Yeah, that's - so myocarditis will lead to cells that become fibrotic and you need fibrosis in the heart, in a normal heart, in order to get fibrillation, in a small normal heart.

<EXAMINATION BY MR JORDAN

Q. Professor Wilde, can you hear me all right?
A. I can hear you, yes.

Q. Thank you. Professor Wilde, I have a question for you, it's a slightly long question but before I ask it, I would like you to consider whether you would prefer to complete the other work that you have in mind before you answer the question. Do you understand?
A. Yeah, but that depends--

JUDICIAL OFFICER: Why don't you ask the question first, Mr Jordan, then.

WITNESS: It depends on the questions.

JUDICIAL OFFICER: Yes.

JORDAN: It does, your Honour, and I'm just being probably overly abundantly cautious, but let me ask the question.

Q. On the basis of your expertise and your review of the materials now available, can you exclude a reasonable possibility that the two girls died as a result of natural causes associated with the CALM2-G114R mutation?
A. I think we dealt with that question earlier. His Honour the judge asked me the similar question. The same question maybe even. I think you cannot exclude it but I consider it unlikely, and maybe even highly unlikely.

JORDAN: Thank you.

NO EXAMINATION BY MS HORVATH, MS LOVE, MR HASTINGS AND DR WATERHOUSE

JUDICIAL OFFICER: Professor, thank you so much for taking the time to talk to the Inquiry and I'm very grateful that you answered the additional questions that we've asked you about. I'm only glad we finished a little earlier than we anticipated. Thank you.

WITNESS: Thank you very much. Have a nice evening.

JUDICIAL OFFICER: And you have a nice day.

WITNESS: Thank you. I think I can leave now?

JUDICIAL OFFICER: Yes, yes.

WITNESS: Yeah, thank you.

<THE WITNESS WITHDREW

AUDIO VISUAL LINK CONCLUDED AT 7.12PM

CALLAN: Your Honour, I should indicate in relation to the affidavit of Karen Hall, it's demonstrated by reference to tab 9-01, red p 5. From the outset the briefing material provided to Professor Wilde included that affidavit but it's no criticism of the huge volume of paper, that particular detail was not raised - we didn't raise it with him specifically and in any event it's now got to be drawn to his particular attention.

JUDICIAL OFFICER: There's no other material concerning that incident, is there?

CALLAN: No. Your Honour, I note the next witness is Dr Calum MacRae who is to be called tomorrow commencing at 10am.

JUDICIAL OFFICER: Thank you. The Inquiry will now adjourn.

ADJOURNED PART HEARD TO FRIDAY 17 FEBRUARY 2023