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TRANSCRIPT OF PROCEEDINGS

INQUIRY INTO THE CONVICTIONS OF KATHLEEN MEGAN FOLBIGG

TUESDAY, 16 APRIL 2019 at 10.00am

PRESENT:

Legal representatives
Gail Furness SC, Senior Counsel assisting the Inquiry

Sian McGee, counsel assisting the Inquiry
Jeremy Morris SC, Senior Counsel for Ms Folbigg
Robert Cavanagh, counsel for Ms Folbigg
Isabel Reed, counsel for Ms Folbigg

Witnesses
Professor Jonathan Robert Skinner, Paediatric Cardiologist and Cardiac Electrophysiologist at Starship Children's Hospital in Auckland, New Zealand (by AVL)
Professor Edwin Phillip Enfield Kirk, Genetic Pathologist and Clinical Geneticist, Senior Staff Specialist in Clinical Genetics at Sydney Children's Hospital and Senior Staff Specialist in Genetic Pathology for NSW Health Pathology
Dr Michael Francis Buckley, Genetic Pathologist and Clinical Director of the New South Wales Health South Eastern Area Laboratory Services at the Prince of Wales Hospital in Sydney
Dr Alison Fiona Colley, Clinical Geneticist and the Director of Clinical Genetic Services for various local health districts in New South Wales
Professor Maria Carola Garcia de Vinuesa de la Conta, Co-Director of the Centre of Personalised Immunology and Professor of Immunology, John Curtin School of Medical Research at the Australian National University in Canberra
Dr Todor Arsov, Senior Research Fellow at the John Curtin School of Medical Research and Centre for Personalised Immunology

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SPECIAL INQUIRY

THE HONOURABLE REGINALD BLANCH AM QC

TUESDAY 16 APRIL 2019

INQUIRY INTO THE CONVICTIONS OF KATHLEEN MEGAN FOLBIGG

PART HEARD

JUDICIAL OFFICER: Yes, Ms Furness.

FURNESS SC: Thank you, your Honour. Did your Honour wish to deal with the potential breach of the non-publication order which I raised yesterday, and I think my friend was seeking instructions?

JUDICIAL OFFICER: Yes. Mr Morris?

MORRIS SC: Your Honour, as I understand the position, the base data and so forth have not been provided to McDonald but there was an opinion sought with respect to the methodology of the genetic experts that had given evidence here, because there was a suggestion made in the report of Professor Fahey, who was the neurologist, to the extent that the genetic testing conducted here had excluded a genetic cause for this presentation, and it was in the context of that report that we sought further expert clarification of that issue.

Your Honour, it is true that we did not approach counsel assisting for the release of that material and for that we express our apologies to the Commission, but it was a scientific issue with which we needed to grapple and we formed the view that it was important to at least provide ourselves with instruction on this issue ahead of this conclave.

JUDICIAL OFFICER: There's not much point in making non-publication orders if they're simply ignored.

MORRIS SC: Your Honour, this was a publication to an expert who had an obligation of confidentiality. It wasn't a disclosure to the public. It was to an expert who owes an obligation of confidentiality and who has prepared a report with respect to this which has helped inform Ms Folbigg of a potential issue with respect to the methodology that's been pursued by some of the experts who have provided reports in this Inquiry, and to that extent it was a legitimate forensic purpose. There has been no further disclosure beyond the provision of that report to counsel assisting and it is from a recognised expert who is aware of the obligation of confidentiality.

Your Honour, I understand the position which your Honour takes in relation to that but, in the circumstances, we extend our apology for the breach but it was for a specific forensic purpose directly relevant to the evidence which has been

given to this Inquiry.

JUDICIAL OFFICER: Well, it would be nice if there were no further breaches.

MORRIS SC: Yes, your Honour.

JUDICIAL OFFICER: And what is the purpose of Dr McDonald's report?

MORRIS SC: Dr McDonald's report really addresses a conclusion which is expressed by Professor Fahey in his report.

JUDICIAL OFFICER: He doesn't mention Professor Fahey in his report?

MORRIS SC: He doesn't specifically but he specifically addresses the postulate or the inference to be drawn from the genetic material that a genetic cause for these children's deaths has been excluded, and it's in that mandatory expression of opinion which is where we've got the problem. In other words, it's the inference to be drawn from the material which is presented to your Honour.

JUDICIAL OFFICER: I would think that I didn't need to be told that. He seems to have assumed that we have embarked upon a process of proving a negative beyond a reasonable doubt. There is absolutely no question that we are not doing that. What we are doing is embarking on a process to find out in the present state of knowledge whether or not there is a possible genetic explanation for the problem. We have got nothing to do with trying to prove a negative beyond a reasonable doubt. The report is completely useless so far as this Inquiry is concerned. You don't have to worry about the fact that the Inquiry is going to find beyond a reasonable doubt that genetic causes have been thoroughly excluded. Is there any other point of that report? Has any money been spent on it by any public funding?

MORRIS SC: Certainly not, your Honour.

JUDICIAL OFFICER: Well, I'm pleased to hear that because it is a completely pointless report.

MORRIS SC: Without seeking to cavil with your Honour's observations, it was a feature of the trial that opinions were given that, because of urine screen testing and so on and so forth, known genetic anomalies had been excluded and that was the state of knowledge as at 2003, and what has been demonstrated without any doubt is the development of genetic understanding in the 15 years since that trial took place and, your Honour, when it is that an opinion such as that given by Professor Fahey is given that genetic causes of these children's deaths had been excluded by this process, it gives rise to a concern, certainly in the minds of those representing Ms Folbigg, that the same submission may be put at the end of this Inquiry.

JUDICIAL OFFICER: I can assure you if the submission is put it won't be received or accepted. We all understand, I am sure, that what we're doing is

looking at what the genetic evidence is available at the present time compared to what happened before, and in 100 years' time perhaps there will be more genetic evidence that will do something else. We are simply doing our best with what we've got at the present time, full stop. What is the possible significance or relevance of the McDonald report?

MORRIS SC: It is to address an opinion expressed in a report which has been submitted to your Honour for consideration.

JUDICIAL OFFICER: All right, I accept that. I don't need it and I will not accept it as evidence in this hearing.

MORRIS SC: May it please the Court.

JUDICIAL OFFICER: But the point that's made in it is a given, which makes it totally redundant.

MORRIS SC: Given your Honour's observations I accept that.

JUDICIAL OFFICER: Yes, thank you.

FURNESS SC: We have Professor Vinuesa and Dr Arsov to be sworn.

AUDIO VISUAL LINK COMMENCED AT 10.16AM

<ALISON FIONA COLLEY, MICHAEL FRANCIS BUCKLEY, EDWIN PHILLIP ENFIELD KIRK AND JONATHAN ROBERT SKINNER, CONTINUING, TODOR ARSOV AND MARIA CAROLA GARCIA DE VINUESA DE LA CONTA, AFFIRMED (10.16AM)

FURNESS SC: Can I begin with you, Professor Vinuesa. Would you tell the Inquiry your full name and professional address?

WITNESS VINUESA: Maria Carola Garcia de Vinuesa de la Conta and I work at the John Curtin School of Medical Research at the Australian National University in Canberra.

FURNESS SC: What is your current position?

WITNESS VINUESA: I am co-Director of the Centre of Personalised Immunology and Professor of Immunology, a group leader of immunology at the Department of Immunology and Infectious Disease, and a scientist of Canberra Clinical Genomics.

FURNESS SC: Your qualifications?

WITNESS VINUESA: I am a graduate in medicine and surgery. I have a Diploma of the Royal College of Obstetricians and Gynaecologists from London UK, I have a PhD in Immunology. I am a Fellow of the Faculty of Science of the Royal College of Pathologists of Australasia. I am a Fellow of the Australian Academy of Science. I am a Principal Research Fellow of the National Health Medical Research Council.

FURNESS SC: Do you have any qualifications in genetics?

WITNESS VINUESA: Not formal.

FURNESS SC: In diagnostic genetics work?

WITNESS VINUESA: No.

FURNESS SC: Are you a registered medical practitioner in Australia?

WITNESS VINUESA: No.

FURNESS SC: So you don't have any clinical role in Australia?

WITNESS VINUESA: No.

FURNESS SC: The laboratory you spoke of, tell me again the name of it, the Canberra?

WITNESS VINUESA: The diagnostic laboratory is Canberra Clinical Genomics.

FURNESS SC: How long has Canberra Clinical Genomics been going?

WITNESS VINUESA: For about two years, but up to very recently it was performing diagnostic assays in a non-NATA accredited form. The NATA accreditation has come only about recently. So we were performing reports on a research basis, which is what we had been doing on the Centre for Personalised Immunology. That has been going on since 2014.

FURNESS SC: So in the last two years you've had NATA accreditation, is that right, or more recently?

WITNESS VINUESA: More recently, it was officially available from this year.

FURNESS SC: Does that mean that you can perform different work now from what you could perform before.

WITNESS VINUESA: Basically it's the same pipeline and the same exercise, but now we do it under formal accreditation.

FURNESS SC: Before that work, that is before 2014 what work did you do of a diagnostic genetic basis?

WITNESS VINUESA: Well we have been running Whole Genome Sequencing since 2009. That became available. We have overseen the Whole Genome Sequencing for over 2,000 individuals. I have overseen the reports and the analysis for most of those, and those include a range of conditions, but predominantly immune diseases.

FURNESS SC: From 2009 where did you perform that work until 2014?

WITNESS VINUESA: Except for the very first year that we did our Whole Genome Sequencing through BGI in Beijing, thereafter we had our own sequencing machine at the John Curtin School of Medical Research. But to save on cost many of the genomes, we have sequenced them through both Microgen in Korea, and through Novogene and Anorad in China for most of the - for the Canberra Clinical Genomics pipeline, everything is sequenced at the John Curtin School of Medical Research, Australian National University.

FURNESS SC: That's been quite recently?

WITNESS VINUESA: We have been sequencing them for two years. It's only the NATA accreditation has become available this year.

FURNESS SC: Have you always carried out the interpretation work since 2009?

WITNESS VINUESA: Yes, mainly as a research scientist. Our primary goal was to identify novel causes of disease, so to uncover novel variants that could be pathogenic.

FURNESS SC: So you haven't been doing it from a clinical outcome perspective, is that right?

WITNESS VINUESA: That's correct.

FURNESS SC: From a research perspective?

WITNESS VINUESA: That's correct.

FURNESS SC: Can I turn to you, Doctor. Would you tell the Inquiry your full name and professional address?

WITNESS ARSOV: Todor Arsov and I'm a Senior Research Fellow at the John Curtin School of Medical Research and Centre for Personalised Immunology.

FURNESS SC: What are your qualifications?

WITNESS ARSOV: I have a medical degree. I have a Masters Degree in Molecular Medicine and both these are from now North Macedonia. I also have Masters Degree in Genetic Counselling from the University of Sydney.

FURNESS SC: I'm sorry, I missed that last one?

WITNESS ARSOV: University of Sydney, and PhD in Biomedical Sciences with project in genetics. Two post-doctoral fellowships in genetic labs as well.

FURNESS SC: Are you a registered medical practitioner in Australia?

WITNESS ARSOV: Not in Australia, no.

FURNESS SC: Have you any qualifications in genetics?

WITNESS ARSOV: Yes I do. I have a Molecular Medicine Masters and Masters in Genetic Counselling, so one's from overseas, one's from Australia, and currently I'm enrolled in the Diagnostic Genomics course through the University, Queensland University of Technology as well.

FURNESS SC: Do you have qualifications to enable you to call yourself a clinical geneticist?

WITNESS ARSOV: No.

FURNESS SC: A geneticist with a pathology--

WITNESS ARSOV: I'm not a clinician. I don't do genetics working or diagnostic or clinical capacity at the moment.

FURNESS SC: Have you ever worked as a clinician, as a medical practitioner?

WITNESS ARSOV: Yes I have, for about five years after I graduated in Macedonia so that was a job, half of the time in the Nuclear Medicine Department and half of the time in an Immunology and Genetics Department.

FURNESS SC: So your role in your position is that of a researcher?

WITNESS ARSOV: That's correct.

FURNESS SC: Turning to the report, Dr Vinuesa, you prepared a report with Professor Cook?

WITNESS VINUESA: Correct.

FURNESS SC: Professor Cook can't be with us today. Dr Arsov can you tell us what role you had in the preparation of that report?

WITNESS ARSOV: Right, so initially I met with Kathleen Folbigg in October 2018 and I gathered clinical information so pedigree and information about the conditions in the family. I prepared the pedigree which I think everybody has?

FURNESS SC: You provided it to me half an hour ago. Is that the document you're referring to?

WITNESS ARSOV: Well I was only asked yesterday, I'm sorry. I was involved in obtaining informed consent for genetic testing from Kathleen as well, and also parental consent for testing her children, obtained the buccal swabs and the saliva sample, which were then analysed. I was involved in analysing some of the variants as well, and in the interpretation of pathogenicity as well.

FURNESS SC: The work you did in taking a clinical history from Kathleen, is that work you've done in respect of other patients or people in your work with the ANU?

WITNESS ARSOV: Sorry, I don't understand. Can you repeat?

FURNESS SC: In your work in taking the clinical history from Kathleen and preparing the tree that you've provided us with, have you done that sort of work in your--

WITNESS ARSOV: At the ANU?

FURNESS SC: --work at the ANU?

WITNESS ARSOV: Yes I do actually. I really have a large collaborative network overseas that I manage, so this is a south-east European network where I liaise with clinicians and record patients, I explore family histories with them and prepare pedigrees.

FURNESS SC: So you've taken clinical history in similar circumstances

recently in Australia?

WITNESS ARSOV: That's right. In Australia it's part of the training in genetic counselling as well. We do a fair bit of clinical training where this is kind of routine work.

FURNESS SC: You said I think you weren't asked to provide this until yesterday or the day before, is that right?

WITNESS ARSOV: That's right, yes.

FURNESS SC: Who asked you to provide it?

WITNESS ARSOV: Amber.

FURNESS SC: Your Honour I might tender that document. I've provided a copy to Dr Colley, but there are further copies.

EXHIBIT #AE REPORT PROVIDED BY DR ARSOV TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: You've said that you were involved in the interpretation of some variants. Were any of the variants you looked at CALM2?

WITNESS ARSOV: Yes.

FURNESS SC: MHY6?

WITNESS ARSOV: Yes.

FURNESS SC: And ID?

WITNESS ARSOV: Yes, IDS, yes.

FURNESS SC: You looked at all of those?

WITNESS ARSOV: Yes.

FURNESS SC: Professor Vinuesa, is there any alterations you wish to make to your joint report?

WITNESS VINUESA: No, but I, I think we did miss variants that the other team identified, KCNA2B, that we think it's, it's interesting and I'm very sorry that I forgot to talk to you about this one in our immediate meeting. I just saw it when I got onto these notes just now, so if there was some time and anybody else wished to discuss this one, I think to us it looks interesting.

FURNESS SC: So it wasn't in your report, but it was in what we call "the Sydney report"?

WITNESS VINUESA: Yes.

FURNESS SC: Are there any other matters in your report you wish to alter or otherwise--

WITNESS VINUESA: No.

FURNESS SC: I tender that initial report.

EXHIBIT #AF JOINT REPORT OF PROFESSOR VINUESA AND PROFESSOR COOK TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: You also provided a letter, Professor, dated 2 December 2018. Perhaps we could have that up on the screen. Do you see that letter?

WITNESS ARSOV: Yes.

WITNESS VINUESA: Yes.

FURNESS SC: What were the circumstances of preparing that letter Professor?

WITNESS VINUESA: When we were approached by the Cardillo Partners and we were asked if we would consider performing Exome Sequencing on this family, we thought that this was something that we did routinely and we were happy to provide that, to do that exercise.

FURNESS SC: The results are as you set out there, two genes were identified?

WITNESS VINUESA: Just on Kathleen Folbigg, yes.

FURNESS SC: What were you told to assume in carrying out that exercise?

WITNESS VINUESA: Possible causes of sudden unexpected death.

FURNESS SC: Was it the only factor?

WITNESS VINUESA: In general, yes.

FURNESS SC: So that's what you were looking for when you came up with those two genes?

WITNESS VINUESA: Yes, also bearing in mind that the majority of the causes would be cardiac arrhythmias so we mainly looked broadly for known genetic causes of sudden unexpected death, particularly those caused by cardiac arrhythmias.

FURNESS SC: Did you do that with a view to any clinical outcome in respect of Ms Folbigg?

WITNESS VINUESA: No, the main question put to us would there be any cause of sudden unexpected death?

FURNESS SC: Sorry, would there be any?

WITNESS VINUESA: Would there be any potential cause of sudden unexpected death, any genetic cause.

FURNESS SC: Based on her DNA?

WITNESS VINUESA: Yes.

FURNESS SC: You came up with those two genes?

WITNESS VINUESA: Yes.

FURNESS SC: And recommended that there be further work done in relation to Kathleen and cardiac investigation?

WITNESS VINUESA: Indeed.

FURNESS SC: When you did this work had you had access to her health records from the prison?

WITNESS VINUESA: No.

FURNESS SC: I tender that.

EXHIBIT #AG LETTER DATED 02/12/18 TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: Coming back to your report, Professor Cook is a Professor of Immunology and Medicine at the Australian National University?

WITNESS VINUESA: Yes, Professor of Medicine.

FURNESS SC: Not a Professor of Immunology?

WITNESS VINUESA: He's a practising consultant immunologist. His title changed when he was appointed as the Professor of Medicine of Australian National University.

FURNESS SC: He's effectively head of the laboratory in Canberra that you work in?

WITNESS VINUESA: He's Director of Canberra Clinical Genomics.

FURNESS SC: He is a registered medical practitioner in Australia isn't he?

WITNESS VINUESA: Yes.

FURNESS SC: His area of particular interest, as with yours, is in immunology?

WITNESS VINUESA: Yes.

FURNESS SC: Thank you. Now, Professor, you set out in the report - and I say "you", although we understand that it's a joint report - the methodology that you followed in doing the work that you did, and perhaps if we can come to page 11 of your report. You understand, from reading the report of the Sydney team, that the methodology followed was largely the same?

WITNESS VINUESA: Yes.

FURNESS SC: And, indeed, the results of the work that you in Canberra did and that was done in Sydney ended up with remarkably similar findings, is that right?

WITNESS VINUESA: Yes.

FURNESS SC: And the only real areas where there might be a debate based on clinical judgment is in the three variants that Dr Arsov indicated before he had an involvement, is that right?

WITNESS VINUESA: Also, the main areas in terms of classification, but I think we also contained the fact of whether we should be looking at variants of unknown significance or not. If we are looking, there would be other variants that we consider, they have a probability of being pathogenic.

FURNESS SC: So, in the event that you were looking at matters that were, let's say, lower than the pathogenicity level in the guidelines, there's only about three or four aren't there, extra genes that you would want to be discussed, is that right?

WITNESS VINUESA: Probably, yes.

FURNESS SC: Yes? So, we're talking half a dozen or so genes that are worthy of discussion, is that right?

WITNESS VINUESA: Yes.

FURNESS SC: Now, the other area, as I understand it, that you consider different from team Sydney is how one should view Kathleen Folbigg's current health, is that right?

WITNESS VINUESA: Yes.

FURNESS SC: Is it the case that it's your view that there has been insufficient medical investigations undertaken, largely if not entirely of a cardiac related nature, to enable you to properly know what her health is? Is that fair?

WITNESS VINUESA: Yes.

FURNESS SC: And you understand that is different from team Sydney--

WITNESS VINUESA: Yes.

FURNESS SC: --who consider that they have sufficient information of her health, leaving aside the Holter test and the stress test which is to be done tomorrow? That's right?

WITNESS VINUESA: Yes.

FURNESS SC: Given that, it may be most useful to turn to those three genes and, in the context of that, Professor, you may indicate where you believe the guidelines should be used and how they should be used in interpreting those genes. Do you understand that?

WITNESS VINUESA: Yes.

FURNESS SC: So, perhaps if I can turn to team Sydney with the CALM2 gene, and I think that we start there, Dr Buckley, with your appendix 2.

WITNESS BUCKLEY: Yes.

FURNESS SC: Now, can we begin with why CALM2 variant was identified by you to form part of this exercise?

WITNESS BUCKLEY: Because CALM2 is a gene which has a known role in cardiac disease and so we - it was a - it - the frequency of the variant in the population was such that it could be admitted to evidence, it was - it was at an appropriate low frequency, it is present in two of the children who had a, a catastrophic event. It was also present in Kathleen.

FURNESS SC: So, that is, it was present in Sarah and Laura, as well as Kathleen, but not present in Caleb or Patrick?

WITNESS BUCKLEY: That is correct, yeah.

FURNESS SC: And the conclusion from that, it was maternally inherited?

WITNESS BUCKLEY: Yes, that is correct.

FURNESS SC: Now, can you take us through your report, starting from the heading "Gene"? I'm assuming, Dr Buckley, that there's nothing you could say that would be meaningful to us about those coloured lines above it, is that right?

WITNESS BUCKLEY: The coloured lines above it purely records that the variant in present in - or illustrate that the variant is present in Kathleen, in the

top row, and in the fourth and fifth row, present in Sarah and in Laura.

FURNESS SC: Thank you. So, taking us through the paragraph that begins with "Gene", if you would, Doctor?

WITNESS BUCKLEY: I would actually think that this, this component of this report was largely prepared by my colleague, Professor Kirk, and I would--

FURNESS SC: Certainly, Professor Kirk?

WITNESS BUCKLEY: --refer you in the first instance for Professor Kirk to address the issue.

WITNESS KIRK: Sure. So, the calmodulin genes, there are three of them, they all encode for exactly the same protein. And that's important because, our view, and the view of the field generally, is that if a variant is present in one of the three genes, that its implications are likely to be relevant to the other two. So, for example, if we see a, a variant in the population in CALM1 that appears to be benign, then it is likely that the same variant occurring in CALM2 or 3 would also be benign.

These are genes that are involved in the regulation of calcium, which is important to cardiac rhythm and cardiac contraction. The clinical features that have been associated with this gene, I think, are quite important and, certainly, many of the people who have been described have had a very severe form of Long QT syndrome, with onset even before birth. So, there have been cases where children have been identified due to tracings conducted during pregnancy as having an abnormality of cardiac rhythm and that this is manifested in the delivery room, in terms of complications of that.

Almost all of the reported variants in all - in all - and certainly in all of those - all of the infantile cases, have arisen de novo. In other words, they have - where, where we have got information, they have not been present in the parents and have only been present in the child, and that's the signature of something that is highly lethal. If we see something that is - that is not inherited, the implication is that people do not survive for long enough to have children themselves.

There are some very rare families - only a handful of families reported, who have a less severe condition, which we talked about yesterday, the catecholaminergic polymorphic ventricular tachycardia, CPVT, and in those families there - so, there are some situations where there are parents who are either affected in a minor way or who are healthy but have abnormalities on stress testing or on other, other provocative testing. In those families, the age of onset of symptoms is much later and the earliest death, I think, is at about four years for any of those, and most of the deaths have been in the range of 10 to 16 years. So, for those - for those particular ones, it's, it's a different condition. It's still a severe condition, but not as severe as the, the more commonly seen situation.

So, I think that's an important difference because we would expect that if someone had a condition that was severe enough to cause death in the first months or in the first year or two of life that that would be one of the more severe manifestations. That's certainly the universal experience in the literature so far. Whereas, if someone were surviving longer term, they could conceivably have the less severe form but we would not expect death in infancy. In particular, the condition CPVT has some particular characteristics and it's a condition - so the catecholaminergic refers to some hormones, so adrenalin and some related hormones, and it is stimulation by those hormones which triggers the cardiac events in people who have that condition.

The way that manifests in people is that the problem occurs in an awake state, typically during exercise but sometimes in response to a startle or occasionally, in strong emotional situations, but exercise. The classic story is of a ten year old who is swimming in a swimming carnival and he sinks like a stone to the bottom of the pool, but there are of course variations on that and I'm sure Professor Skinner may have comments about that as well.

The, the genes have a particular characteristic, which is that they have four - what's called - "domains", EF-hand domains, and within those domains there are binding sites for calcium, and those are important. There are - there are four sets of those, two at one end of the protein, which do not - so far - have not so far been reported as being involved in disease, and two at the other end, which have been reported as being involved. And most of the - all of the, the variants associated with the more severe form of the condition have been in those regions where the - where the calcium sensing occurs. There have been just two variants outside the calcium sensing region, and they've been associated with more variable and the less severe forms of the condition.

The variant that was identified in this family falls outside that region, so there's been a bit of dispute about this between the Sydney and the Canberra teams, but we've looked at this very carefully and it falls just outside and it is in a region where there is some benign population variation. So, there are several variants between - so, there are three variants in the population between where this variant lies and the nearest calcium sensing region, and another in the other direction towards the, the last of the calcium sensing regions.

It's also an amino acid that is in, in proteins within these domains, relatively variable and, even within this protein, at that position, there are three different situations; there is two glycines and another, another amino acid - which I haven't got the right version up in front of me - and then--

WITNESS BUCKLEY: A methionine.

WITNESS KIRK: A methionine. And then, at the end of the protein, this, this, this amino acid does not exist at all. So, we can conclude from that that we don't have strong evidence that this glycine is of important function. That's not the same as saying that a change in that glycine could not produce a disease state, it's conceivable that it could. Sometimes we see a situation where there are chemical changes in one part of a protein that impact the function of

another part. But what it says is that, we can't apply the criterion that says this is in a known functional domain without benign variation, because it's not in the functional domain and there is nearby benign variation.

When - so, so the - so far I've addressed the clinical scenario, which rarely is inconsistent with the pathogenic variance that have been observed in this gene, in that, as we've heard from Professor Skinner yesterday, Kathleen Folbigg does not have clinical features that would be consistent with any of the known manifestations of the condition. It is true that we could not exclude the possibility of CPVT in her without additional testing, but then that would not be consistent with infant deaths in the family because it's a less severe form of the condition, and also, as I say, associated with death while awake, usually during exercise.

When we go down further, there is also some discussion between the groups about the status of conservation of, of this - of this gene, and it is a gene where there is a high degree of conservation in general. But, as we've seen, there is--

FURNESS SC: You need to explain that, I think.

WITNESS KIRK: I'm sorry. One of the comparisons that gets made is with other organisms. And so, during the course of evolution there are changes in, in the protein structure and that's why I'm different from a fish, for example, or a - or a plant, or a worm, that there have been differences in our proteins that have arisen over the millions of years of evolution.

If you see a protein that has changed very little during evolution, one of the implications of that is that, that, in terms of reproductive fitness, those changes are not well-tolerated. That, functionally, it's important on the whole for, for the protein to be as it is. We know that there are very highly conserved proteins that have stayed virtually unchanged through evolution. That will still allow you to have occasional substitutions and this is an example. There have been, I think, 30 or 40 different apparently benign substitutions among these three genes. So, knowing that something is highly conserved does not mean that any change must be disease causing, but it's one of the things that's considered. And it's also, when we move to the computational assessment of disease causation, one of the factors that many of the different programs incorporate in their analysis.

So, looking at the different criteria that we applied when we considered this, this protein, I, I - if we look at the top there, we talked about the gene, the clinical review I've discussed, conservation of the - of the protein. Then there's some discussion of the actual change in the - in the amino acid. In silico tools, these are the, the software tools we used, which are not very strong evidence, they're considered supportive. Those are supportive, the 21 out of 24 tools supporting this being pathogenic. If we go to the next page--

FURNESS SC: Just before you do that, you say that the variant is absent from gnomAD, ClinVar and HGMD databases--

WITNESS KIRK: Yes.

FURNESS SC: --and doesn't appear to have been reported previously. What does that tell you?

WITNESS KIRK: Well, it's, that its status is - it - the fact that it's absent from a gnomAD means that it's very rare. The fact that it's absent from those other databases says that no one has reported it in relation to any disease.

FURNESS SC: Thank you. So we're over the page.

WITNESS KIRK: So at the top there you have that listing of the different, of the different tools and their, their cause. Then there's an image of the, of the protein and below it you can see that the, that the glycine is located in between. So in, in red, blue, green and yellow are the locations of the known disease-causing genes and you can see that there's, there's a gap and there's some population variation there. There is an additional one that I think wasn't, that hasn't been included because it was only added to the database quite recently, I expect.

FURNESS SC: Just slow down a minute.

WITNESS KIRK: Sorry, yeah.

FURNESS SC: You said before that there was some issue as to where something was located?

WITNESS KIRK: Yes.

FURNESS SC: Tell us from this diagram with the red, blue and green--

WITNESS KIRK: Sorry, the, the red box--

FURNESS SC: Wait a second Professor. Just wait till I--

WITNESS KIRK: Right, sorry.

FURNESS SC: --finish my question.

WITNESS KIRK: My apologies.

FURNESS SC: You've said to us that there's some issue as to location.

WITNESS KIRK: Yes.

FURNESS SC: You need to explain to us on the basis of this document--

WITNESS KIRK: Right.

FURNESS SC: --what it is that is located, where it is--

WITNESS KIRK: Yep.

FURNESS SC: --and what difference that makes.

WITNESS KIRK: So the debate is not about where the, the change is. We agree that this particular glycine is affected. The debate is about whether that glycine lies within the functional domain, and we've reviewed the evidence and it is not.

FURNESS SC: So does this diagram tell us that or not?

WITNESS KIRK: This diagram does not, no. We have, we have another diagram that would show that but--

FURNESS SC: So what does this diagram tell us about what's a matter for debate?

WITNESS KIRK: This is mainly, this is mainly about the distribution of known variants. There, there is an image - so the, the, the grey swirly thing with the colour on it and the N-lobe and C-lobe is a visual representation of where the known disease-associated variants sit in relation to the protein, and then below that you see CALM1, CALM2 and CALM3 and the, that's the amino acid sequence of those three proteins, and above the lines are the coloured dots that show reported disease-associated changes in the gene. Below the line are grey dots that show reported population changes in the gene. The red box shows the location of the glycine in all three of those proteins which, as I've said, are structurally identical to each other, and the point that's being made is that that sits in between where the known disease, where most of the known disease-associated mutations, variants lie and that there is some benign variation in, in that region.

FURNESS SC: And how do we apply that to the Folbigg family?

WITNESS KIRK: So that is, that's relevant to interpretation of the variants. So, so one of the, one of the criteria, PM1, refers to a variant being located in - what is it - PM1 refers to a variant being affected in amino acid that's located in a known critical functional domain or hotspot without benign variation and, as we've said, as I've said, it's, it's not in the functional domain. It's not in a hotspot and you can see that quite clearly there are hotspots and they are to either side of where this sits and there is benign variation.

FURNESS SC: And by PM1 you're referring to the ACMG guidelines under "Pathogenicity" and "Moderate"?

WITNESS KIRK: That is correct. So our view is that it is not a - that the characteristics of this variant are more similar to reported benign variation then to pathogenic variation in relation to its location in the protein and that, thus, it is not appropriate to apply that, that PM1 standard.

FURNESS SC: You also refer to PM2 in your summary. That's absent from control. What does that mean again?

WITNESS KIRK: Yes. That means that this particular variant has not been reported previously in health population controls.

FURNESS SC: And PP3?

WITNESS KIRK: PP3 is supporting evidence that, that says that these in silico predictive tools are broadly supportive of this being a pathogenic variant.

FURNESS SC: And BS2?

WITNESS KIRK: BS2, so this, this relates to Mrs Folbigg's health and, as we heard from Professor Skinner yesterday, there is no evidence that she has Long QT syndrome or another recognised disorder of cardiac conduction. While it is conceivable that she could have CPVT that has not yet manifested, that would not be consistent with the severe infantile form of the condition. So you, you either can have one or the other but you, but as far as we know you cannot have both, and there seems to be really quite strong evidence to support that assertion. So we say that the presence of this variant in someone who is alive and well in their early 50s is strongly against this being a variant of the type which causes infantile deaths. The two things are not consistent and that, of itself, represents strong evidence against pathogenicity.

FURNESS SC: Professor Skinner, do you want to add to that?

WITNESS SKINNER: Yes, thank you. I, I, that's a really excellent summary of the papers that I've also been reviewing. I, I will add a couple of things, that Calmodulin hasn't appeared amongst studies of SIDS victims. There's been large studies, reasonably large anyway. One of the most recent was over 400 SIDS victims, a, a collaborative study between the Mayo Clinic in the UK and Calmodulin didn't make an appearance in that, where as some of the other genes that I mentioned yesterday, sodium channel disorder, SCN5A, KCNH2, they did make an appearance.

So despite the fact that this does unequivocally, on occasion, produce very severe life-threatening arrhythmias, they usually present in an awake infant so that it's a witnessed type of collapse, often very early on after birth in a de novo case and they don't seem to present in an infant who's quietly asleep.

FURNESS SC: As you understand it, the four infants in question were quietly asleep?

WITNESS SKINNER: Well, my understanding from the circumstances were that they were found asleep, yes.

FURNESS SC: Dr Colley, was there anything you wanted to add?

WITNESS COLLEY: No, I agree with all that's been said.

FURNESS SC: Professor Vinuesa, in your initial report, or the Canberra initial report at page 20, you begin your discussion of CALM2. Professor, I invite you either to take the Inquiry through your thinking or deal with what team Sydney have said. We're in your hands.

WITNESS VINUESA: Okay, so if we can review our own scoring criteria to perhaps try and explain why, in some points we score differently, so this would be the following page, 21.

FURNESS SC: So this is your scoring as against the ACMG guidelines?

WITNESS VINUESA: Yes.

FURNESS SC: Yes?

WITNESS VINUESA: Okay, so I think first of all I would like to make a point, which is a general point regarding scoring of all of these variants. We do not have enough information to be able to score some variants so, even though we have said no, for example, against PS2, whether this is de novo, if Kathleen did have a related syndrome, let's say CPVT, because we don't have information from her own parents we cannot establish if she had a de novo mutation. So that is already a limitation that we are facing with some of these scorings.

If we go to PM1, "Is it in a functional domain?" normally what we tend to do is go to one of the databases that identifies functional domains. One of the most accepted ones is UniProt. The link is in that page. If we go to UniProt it clearly states that's the EF-hand 3 domain which is a recognised functional domain, spans amino acids 81 to 116, and this variant occurs in 114. So, again, this is subject to interpretation. I admit, at different laboratories, we look at things differently but, in our view, this sits in a functional domain.

FURNESS SC: Just let me stop you given that that's a quite technical point. Professor Kirk, you dealt with this earlier and it's the view from the work that you did that it did not sit in that domain; is that right?

WITNESS KIRK: Yes, that's correct. The, the UniProt positioning is, is quite broad and we've reviewed the consensus sequence, the EF-hand domain section work that Dr Buckley did, and the, this glycine falls outside, just outside but clearly outside, the EF-hand domain and well outside the calcium-sensing area.

FURNESS SC: Is that a matter of clinical judgment?

WITNESS KIRK: No.

FURNESS SC: Do you accept that Professor or not?

WITNESS VINUESA: Yes, it's a discrepancy in interpretation.

FURNESS SC: In interpretation as opposed to a matter of fact, that's what you're saying?

WITNESS VINUESA: Yes.

FURNESS SC: So PM2?

WITNESS VINUESA: So PM2, yes, and I would like to emphasise the significance of absent and rare in healthy databases is a sign of pathogenicity, so it's, of course, a yes against that one. Now we can go on to PP1. PP1, consideration of family, again this is one of the problems that we face with all of these scorings, we do not have full information from Kathleen so, again, we could argue that she could be, first, affected and suffer from CPVT and she just hasn't yet displayed clinical manifestations.

On the other hand, and this is very important and has not been considered, she could be non-penetrant and that doesn't make this residue benign if she's not affected. Non-penetrance is extremely common in cardiac conditions. In fact, it tends to be the norm rather than the exception for some cardiac conditions. So the fact that she hasn't displayed a phenotype and that she might be completely healthy doesn't rule this variant out. So for us again we cannot score it properly. You could assume that she could be affected because of the clinical history of several syncopes. If all of the investigations come back negative we would say she isn't, but at the moment there's uncertainty.

In PP2 again there is a low rate of benign missense variation. When we checked this in the database, the benign variants, we could not find any variant in this region in the four first exomes. If this benign variance exists it would be very informative to know the frequency because if it's a few alleles in the general population if we are dealing with the frequency of one in 30,000 or extremely ridiculously low frequency, I think it's important to discuss those things. So, in our mind, because we didn't find benign variants in the first four exomes, and it is accepted now that you could look at regions of genes instead of entire gene for assessing the, this type of criteria, we think still it met the criteria for low rate of missense variation.

In think we agree with PP3, in silico damaging multiple lines in terms of phenotype specific for disease. I think we have to bear in mind there's been variable expressivity of many different permutations. We know already there are four different phenotypes, CPVT, IVF, Long QT syndrome, sudden death. There's been sudden death in children as we have just heard, five year old while playing. There are sudden unexpected deaths in family, in children with, about nine to ten years of age, and we are learning that each mutation might cause a different effect. So the fact that this mutation has never been found, we don't know if this could cause a phenotype similar in the spectrum that we are looking at. So we still score these as a possibility for sudden unexpected death in infancy.

The other criteria that I think it would be good to review later if there's an opportunity is that these criteria have been designed to identify only monogenic causes of disease. The moment that we have two genes interacting with each other, or two genes that could together work to cause a potential death, then many of these criteria would be changed. For example, the frequency, we wouldn't have to expect such ultra-rare frequencies for each gene. So, again, I think we have to be open to the possibility that it is not acting alone, that the more catastrophic manifestation could be the influence of another gene.

So, with that, I think that some of the discrepancies are a matter of interpretation, which is what typically occurs with ACMG guidelines. In the best of hands we know that laboratories differ, the concordance rate. Even after some type of consensus, there is no more than 80% concordance.

FURNESS SC: So your conclusion is that it's either a variant of uncertain significance or likely pathogenic, that's right?

WITNESS VINUESA: No, we submitted an amended report the day after we submitted this one and the - we had got - the, the conclusion what it, that it was, that it was likely pathogenic.

FURNESS SC: Are you talking about your supplementary report?

WITNESS VINUESA: No, the, the day after that we submitted this one I checked and I realised that this was wrong because we had enough criteria for likely pathogenicity so I submitted that report to Amber and I thought she had circulated it as our amended report. That was the only amendment in our resubmission.

FURNESS SC: So the document on the screen says VUS or likely pathogenic. You're saying that's not the ultimate report that you submitted?

WITNESS VINUESA: Yes.

FURNESS SC: So the ultimate report was likely pathogenic?

WITNESS VINUESA: Yes.

FURNESS SC: The reason for the change from the first report to the second report was?

WITNESS VINUESA: I, I had just failed to amend the label but the classification was correct.

FURNESS SC: So the only difference is the VUS, all the criteria--

WITNESS VINUESA: Information is correct, yes.

FURNESS SC: Is it the case that if you were to assume Professor Skinner's view from a cardiac perspective in relation to Kathleen was correct, just for the purpose of my question, would that then reduce your score to VUS?

WITNESS VINUESA: Not necessarily because I insist we have to consider this could be non-penetrant.

FURNESS SC: Let me stop you there. So your acceptance of the evidence in relation to Kathleen's health is one factor, but not a complete factor in your view that it's likely pathogenic, is that right?

WITNESS VINUESA: If we are saying the same thing, I accept she might not be affected, and I think more cardiac testing will tell us one way or the other. Even if she was not affected, this could still segregate because there are non-penetrant carriers of these conditions.

FURNESS SC: Let me come back to you. Perhaps Professor Skinner did you want to address that particular issue?

WITNESS SKINNER: Yes, of course Professor Vinuesa is correct, that all of these inherited heart conditions have variable penetrance and sometimes expression. But the penetrants issue really is covered quite well with the literature on this gene. It's - degree of penetrants, its likelihood of causing disease has been in the literature to date related very much in the way that Dr Kirk said, if it's a de novo mutation you have severe disease which might cause a lethal outcome in the first year or two. If you have familial disease, there's not a single case of a sudden death under the age of two. All of the deaths produced in the literature have been over the age of two and the majority of those are in teenagers or above during exercise, while awake, not while asleep.

So not only is the phenotype wrong, but the penetrance issue I think really is covered by the literature so far. Is it possible that there would be some - that this particular variant might behave differently? It's stretching credibility but I imagine it's not impossible, but it doesn't follow the pattern of disease established to date.

FURNESS SC: Thank you Professor. You heard what Professor Skinner said about the literature and the age of children and the nature of the activity they were undergoing when they had the experience. Do you accept that?

WITNESS VINUESA: I accept it but I would like to point that there has only been three mutations outside of the calcium coordinating residues and surprisingly each of those has presented with different phenotypes. We had IVF in one, sudden unexpected deaths in other, CPVT in another one. Different ages affected. We have an unaffected published individual, the mother of the pedigree that I submitted for that CALM1 mutation in the F90 residue. So who is going to tell us that this next residue is not going to come with slightly different phenotypes, slightly earlier age of onset? So I perfectly agree with you, that from the published cases they have tended to be either

late onset for the inherited ones and early onset for the de novo ones. But again this could be de novo in Kathleen as she might be non-penetrant like the mother in this pedigree.

I think eventually it will be a laboratory testing these variants that will give us the result and this is not that difficult to do, so I mean it is conceivable that we could put this to the experts in CALM2 and ask them to bring us back a valid assay that could tell us if this mutation is pathogenic or not. So I think on the basis of this information I would not feel comfortable with excluding its potential for pathogenicity.

FURNESS SC: So it's a potential rather than something that's being published to date, is that correct?

WITNESS VINUESA: All of this we are reviewing is analysing the potential for pathogenicity. This is a novel variant. Precisely because they are novel the potential for pathogenicity is greater than those that might be already known, except for those proven pathogenic.

FURNESS SC: You're giving significant weight to the fact of that potential lending you to be more conservative in your interpretation, would that be right?

WITNESS VINUESA: Well again it's where in the spectrum are we placing the acceptable uncertainty? ACMG criteria focuses on the 1% to 10% uncertainty only in order to call it pathogenic or likely pathogenic. Is this what we are after in this exercise, which is another concern that we might have? I mean, could we cope with a 15% uncertainty?

FURNESS SC: What's your level of uncertainty in relation to CALM2?

WITNESS VINUESA: I don't think we can give it a number. I think none of this can really be accurately measured. I think on the basis--

FURNESS SC: If it's likely pathogenic, isn't it then in the 1% according to ACMG?

WITNESS VINUESA: It would be the 10%, likely pathogenic is 90% probability of being the cause of disease.

FURNESS SC: And pathogenic would be 1%?

WITNESS VINUESA: Exactly, 99--

FURNESS SC: So you're putting this in the 10%?

WITNESS VINUESA: According to the, to our interpretation of the criteria at the moment, yes with some considerations. We are not sure yet whether the mother is affected. We do not yet know if - and we don't know for certain if the children had this phenotype. We are taking some assumptions that they died from a sudden unexpected death. So again this is part of the problem we

have, we have with ACMG criteria, that we have to make some assumptions. So it is impossible to give it a precise number.

FURNESS SC: Professor Skinner, in terms of the assumptions that you have made, using Professor Vinuesa's language, you have formed a clinical view I take it based on the medical evidence available to you from Kathleen and the children, is that right?

WITNESS SKINNER: Yes I have. I would also - I think this is, you know, a healthy discussion about a difficult subject, and I think - I would like to just take some issue with the PP4 there in the criterion where it says phenotype specific for disease. To my knowledge this condition, Calmodulin, has never been linked to SIDS. I accept that I have never - not read every paper, but I believe I would have known if Calmodulin has presented in SIDS. I do accept that it causes sudden cardiac arrest in infants, but to date there have been witnesses because the children or the infants were awake. So SIDS is a specific phenotype, you know, the children are asleep in bed, and so I think to say that that is supportive is possibly stretching the criterion.

FURNESS SC: Professor Kirk, would you agree with that?

WITNESS KIRK: Yes, I, I think so - well I've got a number of comments but I'd agree with that statement, yes.

FURNESS SC: Do you accept that in relation to SIDS Professor Vinuesa?

WITNESS VINUESA: Look it's difficult to know. As I say, I myself don't know if every case was really reported to be wide awake. It definitely is a case of sudden unexpected death in the young.

FURNESS SC: So you agree with what Professor Skinner has said in terms of what is known, is that right?

WITNESS VINUESA: With some hesitation because I haven't read myself about all of these individual cases, so I can't say for sure that they were all awake and I assume that he is correct and he knows the literature.

FURNESS SC: Assuming that Professor Skinner is correct, would that then remove your SIDS yes from PP4?

WITNESS VINUESA: It wouldn't because we are still dealing with sudden unexpected death. We have not had electrocardiographic assessment from three of the children. We have had from - we definitely don't have from Caleb and Sarah - sorry, this was present in Sarah and Laura. We did not have any CT from Sarah and Laura's, according to Professor Skinner it was not, it wasn't even diagnostic nor he could exclude a channelopathy on the basis of that ECG because it was poor quality. So I don't feel confident we can exclude this phenotype as being inconsistent with the form of death of the children.

FURNESS SC: Is it the question of excluding or rather the weight one gives to

it?

WITNESS VINUESA: Look it's only in semantics. I, I think we cannot say it's not consistent with the presentation because there is variable expressivity in all of the syndromes. We've just seen it even for CALM2, four different disease presentations. So I just think we would be stretching it saying that it cannot be the cause of SIDS, if it can cause CPVT, IVF, sudden unexpected death and Long QT syndrome.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Do you want a detailed response or just to that point?

FURNESS SC: You give the response you wish to give bearing in mind time.

WITNESS KIRK: Okay. So--

FURNESS SC: I'll leave it to your judgment Professor.

WITNESS KIRK: Right. Can I go through the different criteria and address those or--

FURNESS SC: Certainly, if you can address it in a way that's meaningful to the Inquiry that would be very useful.

WITNESS KIRK: I'll do my best. So in relation to the comments about de novo variants, the reason that finding a variant that is not present in the parents but is in the child is a strong piece of evidence, is precisely because of severe conditions that are not present in healthy parents. In this case it's true that we can't say that Kathleen has - whether she has a de novo variant or not, we don't have her parents, but she does not have a severe condition. Her children do not have a de novo variant, we can say that with certainty. So there's no basis for even considering that that is an issue. The lack of information about Kathleen's parents does not prevent us from accurately assessing that piece of information.

With regard to the EF-hand domain I would defer to my colleague to my right, but this is not a matter of opinion, it's a matter of fact. The variant is not in the domain. We agree on the PM2. In terms of co-segregation in the family, the way that this works in practice, for those of us who are working in diagnostic genetics, is this. Supposing I have a family with five or six individuals who are affected by a specific syndrome. I know they're all affected. In one of them I identify a variant that I think might be the cause of that disease. I then test the other four to see whether that travels with the condition.

So you need a clear diagnosis of a condition in the people that you're testing to even consider applying this criterion. Kathleen has no condition. There is no basis on which you could possibly apply this criterion in this family. I think that it's really - I don't have a strong objection to applying PP2, I think that's reasonable. We agree on PP3. The criterion for PP4 is patient's phenotype or

family histories highly specific for a disease with a single genetic aetiology. So we have a specific condition that has one known cause, or at most two or three. We test a person who has that condition, clearly has that condition, usually a very rare condition, for that one gene. We find a variant, and that's supporting evidence.

But SIDS certainly does not fall into that category. So there is no way of applying that criterion. It simply cannot be done. So I think that leaves us with PM2, absent, rare in healthy databases, and the low rate of benign in its variation and in silica damaging which under the criteria falls as a variant of uncertain significance. Then we come to the clinical assessment and all I can say is that yes, we do see variation in genetic disease, but in the de novo cases related to this condition, which are the ones that have presented with severe disease, we do not.

And yes, it is conceivable that there could be something that is completely outside the experience we've had so far, but I think we're addressing current knowledge and within current knowledge I think I feel quite strongly that we can apply BS2, which makes this a variant of uncertain significance, because we've got conflicting evidence, and one where the weight of evidence is against it being pathogenic, but I accept that there are limitations to our knowledge and it's not inconceivable that this could prove to be relevant to the death of two of the children. I think very unlikely but not inconceivable.

FURNESS SC: Thank you. I think we've exhausted CALM2 your Honour. Can we move then to MYH6. Starting again with you either Professor Buckley or Professor Kirk, or Professor Skinner, or Dr Colley, who would wish to start with that variant?

WITNESS BUCKLEY: Again I think this variant has been particularly analysed by Professor Kirk given his cardiac genetics background, so I will ask Professor Kirk to address the question if that's appropriate.

FURNESS SC: So we're at appendix 8 of the Sydney report.

WITNESS KIRK: All right, so, so this goes to the--

FURNESS SC: Just before you do, Professor--

WITNESS KIRK: Sorry, yep?

FURNESS SC: --we understand that this--

WITNESS KIRK: Yes.

FURNESS SC: --has been amended, although not amended on the document on the screen, to exclude Patrick.

WITNESS KIRK: Yes.

FURNESS SC: So that we all understand the document. Yes, Professor Kirk?

WITNESS KIRK: Yep. So, interpretation of this variant, the starting point has to be understanding the relationship between the gene and a relevant condition. So, I, I mentioned yesterday the framework for gene phenotype associations. Now, there is a great deal of evidence linking changes in this gene to congenital heart disease and also to cardiomyopathy. But we know that the children do not have congenital heart disease and nor does Kathleen and there is no evidence of cardiomyopathy on any of the post-mortems. In any case, it would not present at this very early age, and there's no evidence on echocardiogram in Kathleen Folbigg of a cardiomyopathy. So, those phenotypes are not really relevant.

It is true that there are some very cases of people with known disease-causing genes - variants in cardiomyopathy genes who have had sudden death, usually in early adulthood, who have not had post-mortem evidence of cardiomyopathy. There are just two or three cases, I believe, in the literature of that, and it's thought that that relates to progressive disarrangement of the muscle fibres predisposing to a problem with the cardiac rhythm that is not yet manifested by thickening of the cardiac muscle. So, there's still a manifestation and it's progressed over a long time.

So, those known conditions associated with this gene are not relevant to this Inquiry. There is no evidence that they are relevant at all. That leaves the question of disorders of cardiac rhythm and there is - there is a single paper - if we could go down, there is a single paper from colleagues here in Sydney actually that associate a variant in this gene with, with a disorder of cardiac conduction, of the passage of electrical signals from the atrium of the heart through to the ventricle of the heart.

FURNESS SC: You might just have to slow down a bit, Professor.

WITNESS KIRK: Yeah, sure.

FURNESS SC: We're actually trying to understand what you're saying.

WITNESS KIRK: I'm sorry. What have I said that's not clear?

FURNESS SC: We just need you to slow down.

WITNESS KIRK: Yes. Okay, my apologies. Okay, so, there's a paper from a group in Sydney in which there was a family - there was a 24 year old man who had a cardiac arrest and his three children had different conditions. One had episodes of syncope - of passing out - from the age of nine months and she had episodes of slowing of the heart rhythm with periods - brief periods of - well, 13 - up to 13 seconds of the heart stopping. She had a pacemaker implanted. There was another child who had periods of syncope from 12 months and another who had several episodes of loss of consciousness from, from three months. So, no deaths, but certainly some worrying episodes.

Now, there, there was testing done in the family which identified a change in the MYH6 gene. At the time, the population databases that we currently use were not available, but we now know that, that this particular variant occurs six times in the gnomAD database, which is rather, rather common for a very rare condition that's not been reported previously, but you wouldn't completely exclude it. I do think that if the authors had had access to that information today, they may have reconsidered publishing the paper, but maybe not.

In addition, the - one of the probands - the affected man's parents, who was completely unaffected and had normal cardiac examination, also had the variant.

FURNESS SC: Are you talking about Lam et al on page 25?

WITNESS KIRK: Lam et al..(not transcribable)..yeah, yep.

FURNESS SC: Perhaps if we could have that on the screen.

WITNESS KIRK: So, that's the only evidence that I can find linking this gene to a relevant condition and the evidence is uncertain. There's no functional evidence presented by the authors of the paper, no really - the majority of the people we know about who have got this variant are not known to have any cardiac problem. We just don't know whether that might turn out to be relevant or not. There haven't been any subsequent reports that are similar.

There is also a paper that - that's quite low quality, that links this condition to a different cardiac disorder called Wolff-Parkinson-White, which is sort of the opposite of heart block, where there's an extra pathway for electrical conduction and, and that's a much milder condition and, again, the evidence is weak. But, again, it's a different condition.

So, I just want to emphasise that just because we know that a particular gene is associated with a cardiac condition, you can't infer that there would be different cardiac conditions. If we knew that a variant in a particular - variants in a particular gene were associated with melanoma, you wouldn't expect that that might also give you acne, for example. They're both skin conditions, but they're different and so you need to separately prove associations with different types of condition.

So, our view is that the evidence linking this gene to any relevant cardiac condition is limited under the ClinGen framework. So, we take the view that in that situation you can't really go on and assess the variant any further against the standards. However, we understand that the Canberra team did proceed to do that, and so we've also looked at the criteria. I don't know if you want to - me to go through those now--

FURNESS SC: Just let me see if Professor Skinner wants to add, firstly, before we deal with team Canberra and give them the opportunity to provide their understanding.

WITNESS SKINNER: I don't think I've got anything really additive. I think Dr Kirk has summarised things very nicely. We've never seen this disease or condition in New Zealand and my reading of the literature leaves me unimpressed of its relationship to sudden death, as that single case report, it's open still. I'm not convinced.

FURNESS SC: Thank you. Now, Professor Vinuesa, you deal with this on page 23 of your report and your scoring is borderline between "uncertain significance" and "likely pathogenic"?

WITNESS VINUESA: Correct.

FURNESS SC: That's not changed.

WITNESS VINUESA: No.

FURNESS SC: Would you like to explain to his Honour the basis on which you have come to a different conclusion from team Sydney?

WITNESS VINUESA: Yes, well, I guess, first of all, we are looking at - we are asking the question not "Is it usual?", but "Is it possible?". We've heard that the phenotype of cardiomyopathies is not relevant to the condition and I refer you to page 5 of our response to Professor Kirk and Dr Buckley, where we quote an expert in the field - I think he is one of the world authorities - Ackerman, in not just sudden unexplained death but autopsy negative sudden unexplained death. He states:

> "A standard for a forensic autopsy may fail to recognise subtle features of a cardiomyopathy or that sudden death may occur with sub-clinical disease. This supports the evaluation of cardiomyopathic genes, in addition to channelopathy genes, even in the setting of seemingly autopsy negative SUD. As evidence, in our cohort, half of the exertion related SUDI cases have a VUS in one of the channelopathic or cardiomyopathic genes that remain stuck in genetic purgatory currently."

In terms of a few of the criteria that we have heard, again, may I say that we cannot score search on criteria like, for example, PS2. In terms of the frequency, these are little frequency. These few alleles still means it's only found in one in 32,000 people, which is extremely low, and we know that these gnomAD databases are not manually curated. We don't know that these are really healthy individuals, they haven't had an ECG. So, still, this very low frequency, to us, doesn't justify excluding this criterion.

There is also the - yes, sorry, there is the issue that has been raised - sorry, I thought - I'm not sure what you are talking about, in terms of the PP4 phenotype specific for disease. And, again, we come back to the fundamental question, where a protein is exercised, assuming that we are going to contemplate an alternative hypothesis from the one that has been put to the other by the forensic pathologies, we are contemplating that there is a

phenotype that causes sudden unexplained death and it could be any of these. It could be caused by any of the genes linked to channelopathies or cardiomyopathy diseases.

Now, if there is not - if there's an assumption that there is no phenotype, this exercise is futile, we wouldn't be looking at any genes associated with these diseases. So, if we assume that there could have been a cause that's linked to one of these cardiac conditions, we have to be flexible with this criteria. We do not have electrocardiograms from either Caleb or Laura, so I do not think that we can completely exclude a phenotype that we are postulating could be possible. It's a sudden unexpected death.

Now, there has also been - it's been raised that there is only probably one peer-reviewed publication in which there are three family members, or three children affected from a very early age with cardiac arrhythmias out of this MYH6 mutation. I mean, Chris Semsarian is one of the leading experts in Australian in sudden unexpected death. I do appreciate that there are limited studies but, again, we come back to the question, is this usual? I agree, there is not enough information. But is it probable? To us, this segregation, with another variant, in a gene that could cause cardiomyopathy, well, it is possible. So, again, it's where are we placing our threshold for--

FURNESS SC: Well, where were you placing your threshold for MHY6(as said)?

WITNESS VINUESA: So, again, we've - because of this two, potentially one, moderate criteria and potentially three supporting, we think it could lie still borderline. And, again, it will depend if, if - I mean, once we have full cardiac assessment of Kathleen, things might change slightly. Unfortunately, we don't have the possibility of going back to the children and doing more exams, which is normally a possibility in routine clinical practice.

So, again, what we are trying to do is different from a routine clinical diagnosis. And, again I would like to say that, there are a few criteria that we can't score as you would score a clinical diagnostic test. We - there are - there's information that's not available here, so we have to be a little bit more flexible. We would still sit on the borderline of--

FURNESS SC: So, around 85 to 90%, is that right?

WITNESS VINUESA: I, I still think it's very difficult to give it a number.

FURNESS SC: Well, it is, but if it's a borderline and one end's 90%, that has to be one end of it, doesn't it?

WITNESS VINUESA: Okay.

FURNESS SC: Now, you referred to Ackerman, the Ackerman paper. Now, can I turn to either Professor Skinner or Professor Kirk, are you familiar with that paper? Professor Skinner?

WITNESS SKINNER: I'm, I'm certainly familiar with the paper, yes. I'd, I'd, I'd have to disagree with - let's put this in perspective. This paper by Ackerman is a discussion about exercise related sudden death in older children, teenagers and youth. So, in this group, inherited heart muscle disorders, cardiomyopathies are - do begin to present, and they are an important cause of sudden death in athletes. In most cases they present in mid/late adult life, in fact, but some present in the teens and the features may indeed be subtle. And when it's a - there's a tragic sudden death during a sporting event, sometimes the, the pathological features are quite subtle. But no-one had - to my knowledge, has ever proven a cardiomyopathy as a cause of sudden unexpected and autopsy negative death in infancy.

The exome studies which have looked at sudden unexplained death in infancy have not revealed cardiomyopathy genes as potential candidates. MYH6, MYH7, even all of the genes that are commonly linked to hypertrophic cardiomyopathy have never been implicated in sudden unexplained death in infancy.

So, I, I think that this would - is really stretching credibility even further. I, I, I certainly accept the point that we want to put up a hypothesis and then together make an assumption or try to establish whether it's possible, but this is really improbable, in my view.

FURNESS SC: Thank you. Would you defer to Professor Skinner's view on that, Professor Vinuesa, given his experience?

WITNESS VINUESA: Yes, with one comment. That, again, many of these cardiac problems manifest for the first time with sudden unexpected death and may be autopsy negative. So, with that, I still think we have to take into consideration variable expressivity and the potential for alternative pathogenic mechanisms.

FURNESS SC: Do you accept that the Ackerman article is about children and young people in entirely different circumstances than the Folbigg children?

WITNESS VINUESA: Well, exercise is a type of stress-induced cardiac event, so I'm not sure it's completely different. We are assuming that there could be stress-inducing conditions that have triggered these cardiac events in the children. So, I - the, the context could be slightly different, but I think the pathophysiology underpinning this article could be related to the family.

FURNESS SC: The children were all found in bed asleep.

WITNESS VINUESA: But we have heard that there were stressors to the heart. Laura had myocarditis, that puts stress. Infections of - put stress of different types to the cardiovascular system. So, I don't think just because they were in bed - and, I mean, young infants spend a lot of time in bed or asleep - we can exclude stressors to the heart. And, again, I defer to the cardiac experts, but I just don't think we can rule out - again, is - are we talking

about completely excluding these possibilities or can we contemplate that there might be some stressors.

FURNESS SC: You understand that Professor Ackerman's article referred to children who were playing, dancing, running, playing football? You understand that, don't you?

WITNESS VINUESA: I do.

FURNESS SC: I think we can put Professor Ackerman's article to one side, can't we, Professor Vinuesa?

WITNESS VINUESA: Fine.

FURNESS SC: Your Honour, I note the time.

JUDICIAL OFFICER: Yes, we'll adjourn for 20 minutes for morning tea.

SHORT ADJOURNMENT

JUDICIAL OFFICER: Yes.

FURNESS SC: Thank you, your Honour. I think we've completed the evidence on MYH6. Is there anything further you wanted to say Professor Vinuesa?

WITNESS VINUESA: No.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: No, thank you.

FURNESS SC: Dr Colley?

WITNESS COLLEY: No, thank you.

FURNESS SC: Dr Buckley?

WITNESS BUCKLEY: No, thank you.

FURNESS SC: Professor Kirk?

WITNESS KIRK: I guess only that if we were classifying the variant there are some points of disagreement on the way the criteria have been applied.

FURNESS SC: That haven't already been raised?

WITNESS KIRK: Yes, so we did not classify the variant because we thought the relationship between gene and condition wasn't strong enough, but if we had done so then we would have used different criteria from those that

Professor Vinuesa has applied.

FURNESS SC: So--

WITNESS KIRK: For similar reasons to the previous variant so - I mean, of course I can go through those.

FURNESS SC: We're talking about page 23 of the Canberra report.

WITNESS KIRK: Yep.

FURNESS SC: Which criteria are you talking about?

WITNESS KIRK: So we wouldn't apply PM1 because there is quite a lot of benign variation. So this is the criterion that says in a functional domain without benign variation, and there is actually a lot of benign variation around the location of this amino acid, including for the amino acid itself there are 28 alleles in gnomAD of a different amino acid change affecting that one.

Similarly, we wouldn't apply the segregation for the same reason as for the CALM2 variant, and we wouldn't apply PP4, the specific phenotype for the same reason as the CALM2 variant.

FURNESS SC: Can we then turn to IDS and Professor Vinuesa you have in your response to comments made by team Sydney, this is at page 5 of that document, indicated that you would defer to a metabolic disease specialist on the question of IDS, that's right?

WITNESS VINUESA: I think it would be good to consult with a metabolic expert for sure.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Well as we discussed yesterday, the biochemical evidence as well as the clinical evidence and to a lesser extent the post-mortem evidence are all strongly against this child having had Hunter Syndrome.

FURNESS SC: Your experience is in metabolics, Professor?

WITNESS KIRK: So I trained for a year in the department that did the biochemical testing in 1996 and I have been continuously involved in the diagnosis of children with metabolic disorders since that time, and in particular I've had an interest in the mucopolysaccharidoses and was involved in managing children with those conditions until about five years ago. So I think I'm in a position to comment.

FURNESS SC: Do you accept what Professor Kirk has said, Professor Vinuesa?

WITNESS VINUESA: With one exception. There are in most of the

recommendations for testing this disease, the gold standard hasn't been applied to this case, nobody has measured I, II S deficiency in the right cells. There are - most of the recommendations I have read and I can print out and give some papers to the Inquiry, and I'll cite one from a paper that I've got here:

> "It should be emphasised that a negative urinary GAG test, even using a quantitative assay, does not necessarily rule out the diagnosis of an MPS disorder. False negative results may occur due to dilute sample", which is not the case here, "Variations in GAG excretion over time and overlap in ranges between affected and unaffected patients."

I mean there are reports that the peak of glycosaminoglycans occurs between ages of ten and 19 in infants. It tends to be uniformly low. I think those are considerations when we look at an extreme phenotype like an eight month old baby potentially suffering from the disease. There have been deaths as early as six months reported in Hunter Syndrome, and then with respect to not having the typical histological characteristics, it would be important to know what has been done. I mean there are reports of death in 7% of Hunter cases from cardiac conduction defects. There are complete atrial ventricular blocks described and in some of these cases the pathology is really fine - could be fine pathology looking at the area of the bundle branches.

Now has this been looked into detail in this child, again it would be good to leave it to a pathologist or a metabolic expert, know whether all this has been assessed properly, but what we are saying is that in essence the definitive diagnosis is doing the enzyme activity tests and it can be confirmed by a molecular assay such as the one we have done identifying a likely pathogenic variant, that is just what I wanted to add.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Look it's true that definitive testing would be enzymology, in practice in the field in a clinical setting like this, if we did the urine metabolic screen, the urine metabolic testing that has been completed, we would not consider that necessary. The main weakness of the assays relates to a different type of mucopolysaccharidoses type III and particularly type IIIB where some of the milder cases could be missed, particularly by the quantitative testing and in relation to cardiac causes of death, this relates to quite advanced disease and it's not in a scenario where you'd have someone who was apparently clinically unaffected.

FURNESS SC: So, you remain of the view that there's no good evidence that Patrick had Hunter Syndrome?

WITNESS KIRK: I do.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: Yes, I've never heard of this condition causing sudden unexplained death, I'm aware that conduction problems can develop in advanced disease, but I've never - I've not been aware of it presenting in this manner before.

FURNESS SC: Dr Colley?

WITNESS COLLEY: I'd agree.

FURNESS SC: With?

WITNESS COLLEY: With both Dr Kirk and Professor Skinner, that is Patrick didn't have signs from a phenotypic point of view of Hunter Syndrome and there was no evidence on the post-mortem in addition to the testing that we've heard about.

FURNESS SC: Dr Buckley?

WITNESS BUCKLEY: Nothing further to add.

FURNESS SC: Can we then turn to--

WITNESS VINUESA: Can I add something.

FURNESS SC: Yes certainly.

WITNESS VINUESA: I would just like to add three lines from three different papers that I can provide to the Inquiry, related to MPS syndromes, cardiac disease--

FURNESS SC: Just tell us the article you're quoting from and the author and the publication and the date thank you?

WITNESS VINUESA: Right I've just summarised them here and I've got them all printed out, is it acceptable to give you the printouts later.

FURNESS SC: Certainly?

WITNESS VINUESA: It's just that I've made my summaries, I've just copied and pasted and I've got all the papers printed but I will have to find which of the papers says what it's going to take me a few minutes, could I read this out to you and then provide the papers.

FURNESS SC: Certainly, perhaps after lunch you can tell us - in fact why don't we do that after lunch, you can then read it, so that we understand the article you're referring to and we can match it up, so we will come back to this after lunch?

WITNESS VINUESA: Then perhaps just the one I have, the article in front of me.

FURNESS SC: Certainly?

WITNESS VINUESA: This is an article by Lindsay et al and it says, "The incidence of unexpected sudden death in patients with MPS is 11%." So potentially these have been described.

FURNESS SC: So that's published where?

WITNESS VINUESA: There is the one I have at the moment here, there is an example, a 25-year-old male patient with Hunter Syndrome, MPS II, died suddenly at home as a result of complete atrioventricular block during Holter ECG examination.

FURNESS SC: Patrick was eight months old Professor?

WITNESS VINUESA: Exactly, variable expressivity is very common in genetics, different ages, different presentation, because of different mutations, different modifier genes, different factors contributing to disease.

FURNESS SC: So, from your opinion, the fact that a 25-year-old died in those circumstances, is something that should be taken into account by the Inquiry considering whether an eight-month-old died or had Hunter Syndrome, is that right?

WITNESS VINUESA: Yes, I agree, I think variable expressivity is very common in all cardiac diseases due to all these factors that we've mentioned, modifier genes, environmental influences, second hits in relevant pathways.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: So that 25 year old gentleman who died suddenly had Hunter Syndrome, so he had advanced disease and he had a tragic sudden death in the context of Hunters Disease, that's entirely different phenotype from a sudden unexplained death in the absence of evidence of Hunters Disease, two entirely different things, this is not about expression of disease, this is about no disease versus disease, in order to die suddenly of Hunter Syndrome, you have to have the Syndrome and you have to accumulate the mucopolysaccharides which have to damage your conduction system and thereby you're at risk of dying suddenly, if you're not accumulating the polysaccharides then you won't die suddenly from it.

FURNESS SC: Thank you, you can provide us with the articles after lunch and indicate which articles they came from. Thank you, Professor. So, moving on from IDS, the next one is LANRLP1(as said), now this was a variant that was identified during the reanalysis of the immunology genes. Now Dr Buckley do you want to deal with that or would someone else like to?

WITNESS BUCKLEY: Again, I think this, patients with this disorder present with a typical clinical syndrome, so I might ask my colleague Dr Alison Colley

to address the clinical syndrome.

WITNESS COLLEY: Certainly, I'd be happy to do that. It is indeed a rare condition, there's not many cases in the literature by any means, but the cases that are there clearly show children affected, some from birth, I should mention that the syndrome or the phenotype, includes a skin condition called dyskeratosis, it includes an arthritis, recurrent fevers and an auto-inflammation with autoimmune anomalies. The certain parts of that phenotype do occur or become evident at different ages but in my reading I found that children are able to be diagnosed or certainly have features in hindsight, perhaps the diagnosis wasn't made at the time, but have features in hindsight, sometimes from birth, with the widespread skin manifestations, some are affected from two months, three months and less than six months.

So although Caleb was very young and I just can't remember whether he had this variant or not, my colleagues will tell me, the other children were of an age, particularly eight months, ten months and 20 months, when we would've expected to see some clinical manifestations if the gene change had produced a protein which had produced a phenotype.

FURNESS SC: Thank you. If we go to page 28 of the Canberra Report, there's reference there that Patrick, Laura and Sarah had it, does that help?

WITNESS COLLEY: Certainly, it is thought to be an autosomal recessive condition, and that is we do expect both parents to be unaffected and then multiple children may inherit both copies of a faulty gene and be affected. So, the fact that there are multiple children affected, but I think do we have a heterozygous change, we do, not a homozygous or not a compound heterozygous change, which means that those three children have one faulty gene but not two faulty genes. In the papers and the literature, you do see this condition in consanguineous families, that is when both mum and dad are from the same gene pool, they have the same blood, quite often they're cousins, one of the central papers on this is a family from Algeria that had double first cousin parents.

So, these parents who are blood relatives are more likely to have the same faulty gene which they then pass to the child who has a double dose of that faulty gene and that equivalent to recessive inheritance. So, what we have here is only three children having a single dose of a faulty gene, not a double dose so the genetics doesn't quite fit and the fact that the children didn't have a dyskeratosis or skin condition manifest in them also doesn't really fit.

FURNESS SC: So, the Canberra report has it as a variant of uncertain significance, you I don't think assessed it in accordance with the guidelines, is that right?

WITNESS BUCKLEY: That's correct, we thought it was irrelevant because it was heterozygous for what is typically a homozygous disorder.

FURNESS SC: Professor Vinuesa?

WITNESS VINUESA: Yes we thought this was quite interesting because it was present in Laura who had myocarditis, myocarditis has been described in ways that homozygous known for this mutation and it has been described in a patient with this syndrome. We are very used to seeing our immunodeficiency patients and auto-inflammatory patients very heterogeneous phenotypes and we know that we will have patients who will present with one, two or all the manifestations of the disease. I think each allele we know could contribute to a slightly different phenotype in mice.

In the, in the beautiful paper by Seth Masters, he showed that mice are also variable, there's different penetrance for each of these particular manifestations. So here we are just keeping an open mind and saying there are mutations that are rare, that are present in all three children in this case one as I said with myocarditis, I did write in the report autosomal dominant and autosomal recessive, so they must either--

FURNESS SC: Sorry I'm just going to ask you to slow down a little bit Professor.

WITNESS VINUESA: I am very sorry.

FURNESS SC: You wrote in your report?

WITNESS VINUESA: In my report page 28 I did write AD or AR, autosomal dominant or autosomal recessive which makes me think and I cannot check it now because I do not have internet here, that I must have found some evidence that there could be autosomal dominant inheritance. We all know that even though one particular gene might be or condition inherited in an autosomal recessive fashion most of the time indicating a loss of function, we can find missense variants that act as dominant negatives and can alone confer equivalent phenotypes. We are trying to keep an open mind, we have classified it as a variant of unknown significance but I don't think we can dismiss it on the basis of not fitting entirely to the phenotype.

We are not - we are simply putting this out there because I think it would an error to just dismiss them, given that also we had some of these children with unusual lung or respiratory infections, one of the children was found to have some type of pathogenic bacteria in the lung on autopsy. This type of inflammatory conditions make the body more sensitive and less able to cope with infection and more prone to inflammation, there was myocarditis in Laura, I mean we don't know, we just don't have the evidence, future laboratory tests might be able to test this variant and confirm whether it's benign or pathogenic.

FURNESS SC: I think you in your response referred to a particular article, have you got your response in front of you?

WITNESS VINUESA: Yes, a cardiac conduction defect and left ventricular dysfunction has been identified in a patient with NLRP1 associated auto-inflammation.

FURNESS SC: Is that the Garrelfs article that you're referring to, it's reference 8?

WITNESS VINUESA: Let me just check that reference.

FURNESS SC: It's the last paragraph?

WITNESS VINUESA: Yes.

FURNESS SC: That's it?

WITNESS VINUESA: Yes that's the one.

FURNESS SC: All I am capable of reading at the moment is the abstract and it says that an "NLRP1 associator auto-inflammation with arthritis and dyskeratosis syndrome is a rare, novel auto-inflammatory disorder, cardiac involvement has not been previously reported" and then it refers to a 12 year old girl with arthritis. Is that the article?

WITNESS VINUESA: This is the article yes.

FURNESS SC: What's the relevance of that article, having read only the first sentence of the abstract?

WITNESS VINUESA: That this is the first description of cardiac pathology in an individual with NLRP1 mutation. Now I think this brings us to a very important point, we are making genetic discoveries all the time, these are very recent, relatively recent syndromes and the more evidence and new cases that are presented, the more evidence we will have to support some of these associations. This is an art, it's in its infancy, we are just learning about potential consequences of some of these mutations.

FURNESS SC: So the fact that it presented in a 12 year old girl in this article, is that what enables you to come to a classification of uncertain significance?

WITNESS VINUESA: No it's not related to the age of onset, it's to the possibility that there can be myocarditis associated with an NLRP1 mutation.

FURNESS SC: But it's this article that enables you to form the view as to the classification, is that right or not?

WITNESS VINUESA: I mean the classification is a variant of unknown significance, we don't have enough information to make it benign, so these would not change our classification, it simply supports the possibility that myocarditis can be a presentation.

FURNESS SC: Sorry I put the question badly. This is part of your reasoning process to come to an uncertain significance classification, this article, is that right?

WITNESS VINUESA: Not really. Without the article we have the mouse evidence. We have a mouse deficient in NLRP1 that develops myocarditis. This is, we are adding lines of evidence but it would not impact on the classification necessarily. It simply makes us think that there is some probability of this mutation contributing to the pathology of at least one of the children. These are rare inflammatory syndromes but inflammatory syndromes typically tend to go with pathologies like myocarditis. That is a potential cause of death in Laura. We've been asked to approach this with the hypothesis, with some type of alternative hypothesis. We've just put this forward as a probability.

FURNESS SC: I'm just trying to understand the basis on which you've come to the conclusion of VUS. There's this article which tells us what I indicated earlier. There's the death in mice lacking in NLRP1 being associated with myocarditis and it's a 2012 article. They're the two matters that--

WITNESS VINUESA: No. No, I'm sorry, it's because this variant is novel so it has never been previously in the literature, and I'll stand to be - that already supports perhaps some level of pathogenicity. We have evidence of pathogenicity on two of our three tools that we use for, for scoring pathogenicity. So according to PolyPhen this is a high score of .985, comes as probably damaging. The highest score is one. From zero to one is this damage. According to SIFT we've got a very low score. For SIFT anything close to zero, SIFT goes from zero to one. Zero is very deleterious or it's suggesting, predictive of being deleterious. Nothing of this obviously is set in stone and it could be wrong.

So we have two criteria that say this could be a pathogenic mutation, together with the fact that it's novel, and it's present in three children, including one that has a consistent haemotype is of some interest and makes us think it's a variant of unknown significance for this syndrome. The syndrome has been associated with the Mendelian disease in humans, therefore - sorry, the, the gene--

FURNESS SC: What is the Mendelian disease?

WITNESS VINUESA: It's a, it's a, it's a, it's a, it's a disease that's usually monogenic and it's inherited in a pattern consistent with autosomal recessive or autosomal dominant inheritance.

FURNESS SC: Does anyone from team Sydney want to comment? Professor Kirk?

WITNESS KIRK: I just want to say that Professor Vinuesa is correct that the condition can be inherited in both recessive and dominant fashions and I guess just to say that the 12 year old girl in the report by Garrelfs et al was someone who had clearly been sick for quite a long time. She'd had a splenectomy. She had her spleen taken out a few years previously and she had the heart problem in the context of a multisystem chronic condition. The

mice that Professor Vinuesa refers to had widespread inflammation through their bodies and the heart was part of that overall picture. But, I mean, I, I think we would agree with the classification of variant of uncertain significance, that there isn't strong evidence to link this to the condition in the children and we would regard it, based on the criterias(as said) of the US as well.

FURNESS SC: Now can I turn back to your report, Professor, at paragraph 6.3? This is Professor Vinuesa's, the team Canberra report. So it's on page 13, 6.3. Have you got that in front of you?

WITNESS VINUESA: Yes, I do.

FURNESS SC: This is your summary of findings in section 6, that's right?

WITNESS VINUESA: Yes.

FURNESS SC: And the first finding is "No known pathogenic or likely pathogenic variants in genes that could explain unexpected death were found in four out of four children;" that's your first finding?

WITNESS VINUESA: Yes, according to ACMG criteria.

FURNESS SC: And the Sydney team agrees with that finding?

WITNESS BUCKLEY: Yes.

FURNESS SC: Yes.

WITNESS VINUESA: Can I clarify this? The same mutation in the four children what this refers to, just so that it's clear.

WITNESS KIRK: Yes, we agree with that.

FURNESS SC: So at 6.2 we've dealt with IDS, CALM2 and MYH6, that's right, and then we come down to 6.3 and you then refer to "A number of variants of unknown significance present in all of the children are considered to have the potential to cause disease, potential to contribute to disease," and then you refer to some of them but indicate there was insufficient evidence to conclude that the variants are either benign or likely pathogenic, but are they matters that nevertheless you would say the Inquiry should consider in looking at the genetic picture of the four children in relation to cause of death?

WITNESS VINUESA: I think at least three of those, yes.

FURNESS SC: Okay, which three?

WITNESS VINUESA: From our point of view DMPK and MXT TS6 and SLC12A9, but I think from the other team, as I mentioned, I think they, they have this other variant in KCNAB2 if I remember, if I am saying that correctly, that I thought was quite interesting as well.

FURNESS SC: So in terms of paragraph 6.3, you're happy for KCNQI(as said), SEMA3A and KAG2 in tab 1 to be left to one side? They're no longer relevant; is that right?

WITNESS VINUESA: I think so. I think Dr Buckley made a very good analysis of the prevalence of the intronic variants and we agree that their frequency is probably not consistent with the phenotype or the putative phenotype that we are exploring.

FURNESS SC: Thank you. We'll come back to those, but coming to paragraph 6.4, there are a number of variants of unknown significance present in only some of the children still considered to have the potential to contribute to disease. We've dealt with NLRP1. Are there any of the other four that you accept can be put to one side as not relevant?

WITNESS VINUESA: Look, yes, I, I mean, it's very difficult. Again we are just talking about probabilities with very vague phenotypes so we don't really yet know very much what we are looking at. Of all of these, CACNA1E is, could be the cause of her channelopathy which are probably more possible causes contributing to a death like the deaths that we are observing. So I would be happy to not consider in more depth the others but perhaps - I don't know what the Sydney teams thinks but we are still dealing with a variant of unknown significance for CACN1E but I don't think we can completely ignore the probability that it could contribute to disease with a level of uncertainty that does not reach perhaps, or the level of certainty that does not reach 90% but a potential contributor.

FURNESS SC: All right, so we've got four variants to consider. Can I start, Mr Buckley, with you? And it may be that this is best done by reference to your response document, and the first one of relevance on that document is the - where do you want to start, Doctor? I'm in your hands.

WITNESS BUCKLEY: DMPK would be--

FURNESS SC: Say that again?

WITNESS BUCKLEY: The variant in DMPK document, paragraph 7.2 of Professor Vinuesa's.

FURNESS SC: Thank you. Yes?

WITNESS BUCKLEY: So we know that DMPK is a cause of myotonic dystrophy. The disease mechanism for this particular gene is an expansion of part of the gene adding small units, one upon another, so that eventually you get a very large expansion which is then toxic to the cell. It's called a toxic RNA mutational mechanism. The known phenotypes associated with the DMPK gene are not caused by loss of function or missense variants in DMPK itself. So we would say that the observation of a missense variant in the DMPK gene does not lead to clinical consequences in this family.

FURNESS SC: Doesn't lead to clinical consequences, is that what you said?

WITNESS BUCKLEY: That's what I said, yes.

FURNESS SC: Thank you. Professor Vinuesa?

WITNESS VINUESA: I accept that the known mechanism of action is through these trinucleotide expansions but we know there are precedents for other diseases, for example, Fragile X syndrome, that is predominantly caused and overwhelmingly caused by trinucleotide expansions where there have been reports - not many but there are at least two separate reports - of missense mutations causing exactly the same phenotype. So I don't think it's unconceivable that we could have a missense mutation causing a phenotype that normally would either be, otherwise be caused by a trinucleotide expansion.

I agree that dystrophia myotonica is, is one of the accepted diseases. There has been a link and, and, and I think you discuss it in your report with Brugada syndrome and this is something that again we agree it's one family, it's not a strong link, but we know this: we are at the tip of all of these discoveries. We cannot exclude yet another family having this type of presentation. So, again, new mutations, we are keeping an open mind. Is this highly probable? Perhaps not. Is there some type of probability? Well, it is unknown. We, we, we just - again, somebody would have to do a functional validation on this, very end, and know whether there would be a loss of function and cause slightly unusual phenotype or predominantly the Brugada syndrome that was seen in the father of this family that didn't have the SCN5A mutation and only had the DMPK mutation.

JUDICIAL OFFICER: Professor Vinuesa, are you saying that that is a theoretical possibility that you're not prepared to rule out?

WITNESS VINUESA: I, I guess that's what I'm saying, yes.

JUDICIAL OFFICER: Are you saying that about all the other matters that you've been talking about, about CALM2, MHY6?

WITNESS VINUESA: Some are stronger. Some we have classified as likely pathogenic, which means that they would have a 90 to 99% probability of causing the disease. This one is still a variant of unknown significance so the probability would be lower and we would be in the realm of the, theoretically contemplating the probability. For others I think the evidence is stronger, if that makes sense.

JUDICIAL OFFICER: Thank you.

FURNESS SC: In relation to his Honour's question about theoretical possibility that you're not prepared to let go, is it the case that the DMPK and the ADAMTS6 and the SCLC12A9 each fall within that category?

WITNESS VINUESA: Look, they do but there are unique things about these mutations. First is that they were present in all the four affecteds. So, to me, that is quite interesting and they could be modifying or exacerbating other cardiac phenotypes. I just don't think we can simply dismiss them. As a researcher when I see these kind of things, you know, this is when we get excited. We think this could be an unusual presentation. We need to test it. They haven't been tested. I just think it would be - I would not dare to dismiss them. It doesn't mean that there's a high probability but there is a probability.

FURNESS SC: So it's a theoretical possibility that each of these may have been relevant?

WITNESS VINUESA: Yes.

FURNESS SC: And in relation to CALM2 and MYH6 you'd put it slightly higher?

WITNESS VINUESA: Yes.

FURNESS SC: And IDS you'd defer to Professor Kirk?

WITNESS VINUESA: Or to the reviews that recommend a different type of definitive diagnosis. I mean, I respect what Professor Kirk is saying and I'm, I, I know that this would be unusual presentation but there are description of unusual very early onset presentations with, with cardiac pathology that gives rise to early mortality in the papers that we might be able to provide.

FURNESS SC: So again there's a theoretical possibility in relation to IDS?

WITNESS VINUESA: Although, because the mutation itself is likely pathogenic, I think the probability is high.

FURNESS SC: Higher than a theoretical possibility, is that what you're saying?

WITNESS VINUESA: I would say so, but it would be good to have a definitive negative diagnosis and I wonder if it can be reached because I understand there are fibroblasts from Patrick available so the enzyme test might be possible and that would be quite useful and informative if we could exclude that on the basis of the definitive diagnostic assay.

FURNESS SC: You suggest in your report that that's one of the further investigations that's needed, the functional analysis, in relation to Patrick to, as you say, help diagnose Hunter Syndrome.

WITNESS VINUESA: Yes.

FURNESS SC: Now if I can turn to the Sydney side of the room, Professor Skinner, is it the case, in your view, that Hunter Syndrome has effectively been

ruled out?

WITNESS SKINNER: Yes.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yes, and we didn't go to the classification of the variant and we disagree with the classification of the variant as likely pathogenic.

FURNESS SC: Just while we're here on further investigations, and this is at page 29, another one you suggest is an "RNA sequence of heart or brain tissue samples of the children, if available, to provide insight as to the pathogenicity of various variants;" do you see that?

WITNESS VINUESA: Yes, I see it, yes.

FURNESS SC: I think all of those variants you've let go; am I right in thinking that?

WITNESS VINUESA: You are right but if I may add something that we haven't discussed is that, you know, even in the best case scenarios, and I think Dr Buckley would agree with me, even in the cases where there are definite Mendelian disorders, so no genetic diseases with perfect mode of inheritance, Whole Genome Sequencing can only identify about 50% at best at the moment, and that is because, in part, we know that many of the, of the causative variance lie in regions of the genome that are known coding for proteins.

And there's increasing evidence that this may be structural variants, perhaps not just the ones that we've put in there that turn out to be quite frequent. But many others that we wouldn't know, so part of dealing with this inconclusive genetic studies is performing an assay, that is sequencing RNA that tells you about transcription across the genome, and whether there might be variants that perhaps are sitting in areas of the genome that regulate the way, you know, transcripts are spliced, are cut to come together and make the coding sequence of a protein. And, you know, I mean some of this type of uncertainties are resolved by performing RNA sequencing and finding unusual transcripts. So again there are additional tests that could be performed if we are not satisfied that we have covered all of the possible genetic causes.

FURNESS SC: Dr Buckley?

WITNESS BUCKLEY: I'm not quite sure where to start. Perhaps - well I certainly agree with Professor Vinuesa that when you study a large cohort of people with presumptive genetic disorders, that at best Whole Exome Sequencing, Whole Genome Sequencing, has a diagnostic rate of 50% odd broadly. The problem with that figure is it has both a numerator and a denominator, that the percentage detection rate is influenced by the cases that are provided as well as the ability to detect. So I'm not sure among the undiagnosed individuals who are provided to my facility at Prince of Wales

Hospital what percent, what is the likelihood that many of those would have a diagnosable genetic disorder because they've actually - whether they have a genetic disorder at all. So I think that's one issue that needs to be put out.

I think the other issue is that if you have a - sorry, coming to the issue about transcription, I think it's possible to conduct such studies on tissues. I think the tissues that are provided have been fixed in formalin for very long periods of time, and all the data we know from tissues which have been fixed in formalin for prolonged periods of time is that they are really very suboptimal for doing transcriptomic studies and I think this has been very clearly illustrated by the activities at Genomic, Genomics England, where they compare the diagnostic rate in a cancer setting between samples that have been fixed and preserved for periods of time in formalin versus freshly available tissues.

The only conceivable tissue that we have got which is freshly available in a sense would be the fibroblasts from Patrick I think. So I'm not sure how many ampules we have of those, but the, there is a possibility that those fibroblasts are already non-viable, that they have been sitting there for a long period of time. When you take fibroblast ampules out of liquid nitrogen and try to revive them, at least in my diagnostic labs experience, that that will fail 50% of the time if they have been in storage for protracted periods of time. So it's not a test I would do without having very, very strong conviction that it would generate likely useful information because then we would lose any residual fibroblasts that are in storage which might be useful in another purpose.

I think also that RNA transcription studies only represent the cells in which you get that information from. So we are talking here about reviving cells which have been taken from skin cells, which have been grown in culture, stored for a long period of time, and then making an inference from skin as to what would be happening in a child's heart and in a child's brain. I don't know that - I'm no, I'm nowhere near as confident as Professor Vinuesa that that would yield diagnostically useful information, not the least because how on earth would you control it? How would you know that the results that you had were meaningful. What controls would you use to control the experiment? To me it would be a, an extremely difficult costly experiment to run based on a number of conjectural hypotheses.

I think my last point would be that really we're here today, and I'll quote his Honour this morning, that we are looking at the evidence at this time, compared with what was available in 2003. We're not trying to speculate out into the end of the time that Mrs Folbigg may be incarcerated for. We are simply looking on today's evidence and knowledge whether there was something that would be exculpatory, and I don't think that, that even Professor Vinuesa would be able to say at this point that the evidence that we have got would be sufficient.

FURNESS SC: Is there anything that you wanted to add Dr Colley in relation to the further investigations that should be done?

WITNESS COLLEY: As a clinician I'm always coming from the point of view

that I have patients in front of me with a phenotype with a genetic illness or with an illness, with a condition, with some dysmorphic features, and so the samples I am giving to the laboratory I'm directing a test because there's some concern I have and that the family has with the people in front of me. And so knowing that that family have children or adults with disability or disease, I'm going to do every test that I think is reasonable to try and give them answers that'll give them prognosis, give them treatment and give them recurrence risk for future children.

Doing further tests here, I'm - I guess I'm really concerned the fact that we don't have a putative condition. If Professor Skinner said look I think these children might have had Long QT or I've got evidence of Brugada, then certainly I think we need to think of the Nth degree of what we can do. But we don't have that here. What we have is children who have a catastrophic event but didn't have a phenotype of a syndrome or a condition or an illness that we could diagnose or was diagnosable before that catastrophic event, taking into account Patrick at four and a half months of course. So I can't see my way forward to - and taking into account what Dr Buckley has said about RNA sequencing and RNA testing - I can't see my way forward to supporting the further investigations that have been mentioned.

FURNESS SC: Thank you. Professor Kirk?

WITNESS KIRK: I have nothing to add to that.

FURNESS SC: You referred earlier to not having full information about Kathleen Folbigg, and in 10.3 you state that she should undertake the full cardiac assessment involving a Holter and an exercise test, which as you know that's happening? You understand that?

WITNESS VINUESA: Yes.

FURNESS SC: In the event that that was, in my language, normal, would that enable you to come to some firmer conclusions than you have today in respect of CALM2 and MHY6?

WITNESS VINUESA: Look, yes and no. First of all it would be ideal that she also had drug, the drug induction of certain cardiac arrhythmias so we know that you can induce arrhythmias that otherwise you cannot mask with certain pharmacological treatments, and I think that's pretty standard, so I think I would add one more test to this list, but of course I would leave it to the cardiologist to comment on that. But then we have said a few times here that there are non-penetrant carriers of pathogenic variants and, you know, penetrance is not an on or off switch. Either you have the disease or you don't, and if you're a non-penetrant carrier you still will have the pathogenic variants that might kill or cause catastrophic events in other members of the family, and we have spoken about some of these cases here.

So again, in the context of cardiac disease, these are so common, these non-penetrant forms, that I do not think that would be sufficient. I mean, we

would be able to change some assessments in terms of labelling her as unaffected, so that would be helpful. But we would still not be able to exclude the possibility of her being non-penetrant.

FURNESS SC: Perhaps over lunch if I can ask you to have a look at the two in particular genes and assuming uneffective, and then you can tell us what your interpretation would be, would you do that?

WITNESS VINUESA: Yes, but may I say that it's not just that we lack information on her. We lack information on the children. We haven't had ECGs, proper ECGs on three of them, so I just still don't know what we can exclude the most common causes of cardiac death.

FURNESS SC: Professor Vinuesa we can't do anything about that.

WITNESS VINUESA: Okay, but I was just listing the things that we do not have that impact this interpretation. We do not have DNA from the father. I think if there was some type of, some type of gonadal mosaicism that would present in the children as de novo, and we are missing all that information. I think that is important because it would immediately change the ranking of some of the variants, or make us look at things that we might have not looked at closely. We do not have information on the parents of Kathleen because they were deceased, so we cannot know if any of their variants would have been de novo in Kathleen. So even if she was unaffected, she could still be non-penetrant and just the fact that the variant was de novo would have made it a much more likely variant in terms of pathogenicity.

That is part of the reason besides not having full phenotypes of the children and operating under a different type of checklist that we normally do for diagnosis, that I do not think that using ACMG criteria is appropriate. We cannot score many of the criteria in this family, so we are having less possibility of classifying these variants as pathogenic.

FURNESS SC: Will you do the exercise I've asked you to?

WITNESS VINUESA: I will.

FURNESS SC: Thank you.

JUDICIAL OFFICER: You've been asked that question and you said yes and no. But the answer that you've given has been all no.

WITNESS VINUESA: The yes part comes from if she is unaffected, we can perhaps change the PP1 in some conditions. We still need to think of the probability of her being non-penetrant. So it would change. We would be able to label her as non-affected, but I'm still not sure that we would end up changing the classification. We can do the exercise.

For me the main concern is that, I mean there are limitations in the way we can score this family using ACMG criteria. This is not a routine box ticking exercise

like we do in a clinical diagnosis. We do not have full phenotypes. We are - we have to assume phenotypes, so if you are assuming phenotypes we can't then just discard them on PP4 and say there were no phenotypes consistent with the disease. We are assuming there was a sudden death, and many cardiac conditions manifest for the first time with sudden death. So to ask--

JUDICIAL OFFICER: Was it your idea to do this test?

WITNESS VINUESA: No.

JUDICIAL OFFICER: Why are we doing it then?

WITNESS VINUESA: Which test, sorry?

JUDICIAL OFFICER: The test that Ms Folbigg is undergoing at the--

WITNESS VINUESA: I think, I think genetics is very useful but I think is not appropriate is using exclusively ACMG criteria to classify these variants. It is not mandatory, it is not law. It is used as an orientation for clinical practice. It is used to take clinical actions. Normally the outcome of some of these diagnostic assays is deciding for example of a woman with a BRCA mutation is going to undergo bilateral mastectomy, because the risks, as I see it, to the surgical procedures can be so high, clinicians want to be extremely confident that the variant is pathogenic.

JUDICIAL OFFICER: Is that your answer as to why we're doing this testing?

WITNESS VINUESA: We are doing it see if there is a genetic, potential genetic cause of disease in these children.

FURNESS SC: Might I intervene? I think there's somewhat cross-purposes. Professor Skinner recommended this test be undertaken.

WITNESS SKINNER: Can I speak to that?

FURNESS SC: Yes.

JUDICIAL OFFICER: Yes.

FURNESS SC: Yes, of course you can Professor. Sorry, I didn't know where the voice was coming from.

WITNESS SKINNER: So the, the idea of investigating Kathleen, and ideally the father, is because this is part of what we would do - we came at this in the beginning where the idea of we would throw technology and medical knowledge as it is now at this case. So if these four children died now, the way we would investigate this would be to investigate both parents, any other surviving first degree relatives, and we would do intense cardiac evaluation for evidence of phenotype. So it is to complete the picture. It's part of the whole investigation. It's not requested because of any particular thing that one saw

on the genetic results or anything else. It is because this is what is recommended best medical practice and has been in Australasia since 2008.

JUDICIAL OFFICER: Thank you.

FURNESS SC: I think also Professor Skinner when this issue first arose at one of the earlier consultation meetings, it was clearly before the genetic data was available and analysed?

WITNESS SKINNER: Absolutely correct, yes.

FURNESS SC: I've got various other mopping up bits and pieces. Can we perhaps take an early luncheon adjournment?

JUDICIAL OFFICER: Yes. We'll come back at 2pm.

LUNCHEON ADJOURNMENT

FURNESS SC: Your Honour I just have two very short matters and then I'll have some various other matters to deal with but that can be after my friend has completed.

Dr Arsov, you visited Kathleen Folbigg and created the tree which is now exhibit AE, do you have a copy of that with you, we can give you one?

WITNESS ARSOV: Yes.

FURNESS SC: Do you see in relation to Kathleen, just have a look at it, underneath her name and age you say "11 to 12 year old fainted in swim race"?

WITNESS ARSOV: Yes.

FURNESS SC: What did she tell you about that?

WITNESS ARSOV: She was in the swimming competition--

FURNESS SC: Just come forward to the microphone.

WITNESS ARSOV: She was in the swimming competition, after swimming for a while she felt unwell and fainted.

FURNESS SC: In the water or out of the water?

WITNESS ARSOV: I don't remember that we went in that much detail.

FURNESS SC: It didn't matter to you, in terms of what you were seeking to achieve through that clinical review?

WITNESS ARSOV: So I wasn't seeking to establish any specific diagnosis, it

was just kind of asking what her health issues may have been that she may be able to report, assuming that there will be a formal cardiac interview and assessment going forward, so I just didn't you know think I should put too much effort into - it was a bit of a you know, complex circumstance.

FURNESS SC: So you can't tell us any more, other than what you've just told us?

WITNESS ARSOV: No.

FURNESS SC: Were you there Professor Vinuesa?

WITNESS VINUESA: I talked to her on the phone after that to deliver the results of--

FURNESS SC: What did she tell you about the swim race episode?

WITNESS VINUESA: Yes she said it was in the water as soon as she finished the race.

FURNESS SC: And did she fall to the bottom of the water?

WITNESS VINUESA: She was - somebody looked after her so I don't know that she fell to the bottom of the water.

FURNESS SC: She fainted and got out of the pool?

WITNESS VINUESA: No, she just told me she fainted at the end of the race and I don't know if she fell to the bottom of the pool.

FURNESS SC: You don't know whether she fainted in the pool or out of the pool?

WITNESS VINUESA: No, she did faint in the pool.

FURNESS SC: Professor Skinner, does that mean anything to you?

WITNESS SKINNER: Yes. I think this is a really important event that we need more detail about, two conditions are quite specific, Long QT syndrome type 1 and CPVT can cause sudden loss of consciousness, particularly while swimming, so the detail of that event actually is pivotal here and so on the other hand of course it is possible to - we need the details of that faint, for example if she sank to the bottom of the pool, was pulled out by somebody and given resuscitation, that's one story, if she'd just won a race, felt a bit dizzy and was pulled out and recovered, that's a completely different story, so the details are really important.

FURNESS SC: Professor, you had a look at an ECG taken of Kathleen on 17 May 2011 overnight for us?

WITNESS SKINNER: Yes, so this presumably correlates to the event when she was evaluated by the Cardiology Registrar at Westmead Hospital in May 2011, and the ECG is from 17 May 2011, it's a poor quality copy but it's good enough to interpret basically, and it's normal, the heart rate is normal at around 90 - no it's slower than that, around 60 beats a minute, the QT intervals are normal, I measured those up with digital callipers overnight, they're normal .41, .39 the upper limit of normal being .46, .47. There's no features of Brugada Syndrome, there's no conduction tissue, no conduction defects, in summary it's completely normal.

FURNESS SC: Your Honour, Professor Skinner has to leave us this afternoon. So I will now sit down and deal with some remaining matters with Professor Vinuesa tomorrow.

EXHIBIT #AH ECG OF KATHLEEN FOLBIGG TENDERED, ADMITTED WITHOUT OBJECTION

MORRIS SC: Given Professor Skinner's difficulties this afternoon, I might just focus on him for the time being.

JUDICIAL OFFICER: Yes thank you.

MORRIS SC: But before I do, just in relation to exhibit AF, and this is to Professor Vinuesa, in relation to your--

WITNESS VINUESA: I understood you were, I thought you were interviewing Professor Skinner, I'm sorry.

MORRIS SC: Sorry just before I forget, at page 13 of your report, at paragraph 6.1 and that's exhibit AF, do you see that?

WITNESS VINUESA: Yes.

MORRIS SC: At 6.1 are you referring to a single variant or a variant in a single gene or are you talking about variants collectively?

WITNESS VINUESA: A single variant in a single gene, present in all four individuals, so the same variant in all four children.

MORRIS SC: Professor Skinner, I might just take you to your evidence yesterday in relation to the ECG that was performed on Laura and this is at page 386 and 387 of the transcript your Honour - and in this regard this is at tab 20 page 47 of the tender bundle?

WITNESS SKINNER: Yes.

MORRIS SC: And this is the one where we were talking about there was agonal rhythm?

WITNESS SKINNER: Yeah.

MORRIS SC: And I think at page 388, you indicated that the primary rhythm problem may have been asystole and that after the asystole, that is the lack of beating, you then get this agonal rhythm thereafter, is that correct?

WITNESS SKINNER: Yeah.

MORRIS SC: And really what you're saying is that well - if you could just explain to me, are you saying that that agonal rhythm keeps running but it's insufficient to generate a heartbeat?

WITNESS SKINNER: That's correct.

MORRIS SC: And at 387 you said that it's your belief and I'll read it to you, "It's my belief that most of those deflections are actually due to chest compressions and that's due to the fact that they run about 100 or 110 beats per minute"?

WITNESS SKINNER: Yes.

MORRIS SC: Is what you're saying, is that the activities seen on that ECG as brought about by the ambulance officers administering resuscitation techniques?

WITNESS SKINNER: Yes I think it's a very difficult strip to interpret, but that was my take on it yes.

MORRIS SC: And is it the case that the agonal rhythm can be extended by resuscitation techniques?

WITNESS SKINNER: Well the agonal rhythm is really a feature of a dying heart, so - sorry I'm getting an awful feedback problem suddenly here, so if I stutter that's because I'm hearing myself very loudly - so an agonal rhythm is a feature of a dying heart and if one is giving CPR and providing some support to the heart then the agonal rhythm may go on longer than if you didn't, if that was your question.

MORRIS SC: And what about the administration of drugs in combination with the chest compressions, would that have the tendency of extending agonal rhythm?

WITNESS SKINNER: Well potentially, basically though the heart is usually at that stage when it's so sick that the ECG looks like that, there is usually very little that can be done to bring the heart back, whether it's adrenaline, CPR or any form of resuscitation, but clearly that is what has to be done in this situation.

MORRIS SC: My question is slightly different, does it have the potential to extend the length of agonal rhythm, chest compressions and medication?

WITNESS SKINNER: Potentially yes.

MORRIS SC: In relation to the ECG test and I'm going to need your help with some pronunciation here, there's a sort of a drug provocation test I don't know whether that's the correct explanation for it but epinephrine and ajmaline?

WITNESS SKINNER: Epinephrine and ajmaline, yes.

MORRIS SC: What's the purpose of that Professor?

WITNESS SKINNER: So if you're asking about what tests might be done on Kathleen, is that what you're alluding to.

MORRIS SC: Just generally, what is the purpose of this, is it a drug provocation test or?

WITNESS SKINNER: Yes, yes so rather like an exercise test might manifest occult disease, sometimes drugs are used to manifest occult disease, so the ECG or the heart rhythm may look normal but when we give adrenaline which is the other word for epinephrine, sometimes we can induce an abnormal rhythm in the same way as exercise testing can. And ajmaline is a sodium channel blocker which can manifest a condition called Brugada Syndrome through blocking the cardiac sodium channel.

MORRIS SC: Just explain, is that an alternative test to an exercise stress ECG?

WITNESS SKINNER: The adrenaline test or the epinephrine test, yes it's an alternative to the stress test, it's less well established in medicine, I believe it's less reliable because fewer people have done it, but some people for example are unable to get on a treadmill and you know, maybe they're infants and we need to do the test under anaesthetic or something like that and so it is still used in some circumstances.

MORRIS SC: Is that - is the provocation test of each of those something that you would leave to the discretion of an electrophysiologist, cardiac electrophysiologist or is that something in the clinical setting that you would direct to take place?

WITNESS SKINNER: Well both but I think in this case Ms Folbigg is going to be investigated by a cardiac electrophysiologist who is fully aware I understand of the circumstances and so he or she would need to make that judgment call, I would say that in New Zealand we've abandoned in particular the ajmaline challenge to a large degree because it's really not specific enough, it is quite sensitive but a fairly recent study shoved it up to 20, 25% of normal people can actually have an abnormal response to this, so we've pretty much abandoned it other than in very specific circumstances.

MORRIS SC: Just to explain that, do you mean that it can trigger a false positive?

WITNESS SKINNER: Indeed.

MORRIS SC: But that's in about 20 to 25% of people you say?

WITNESS SKINNER: Well there needs to be further study on that but this was a research study of - from memory I could produce the paper for you if you like - but it's around a hundred people who have a different sort of arrhythmia called SVT which is much, much less dangerous rhythm, a common heart rhythm disturbance that's usually treated with medications or can be cured. But they did a research study giving ajmaline and to their great surprise 20 to 25% actually had a positive result for Brugada syndrome, and the editorial that went with that said something like does everybody have Brugada syndrome.

MORRIS SC: Would you be able to provide us with a copy of that paper at some point?

WITNESS SKINNER: Of course, yes.

MORRIS SC: In relation to heart conditions, is it fair to say in simplistic terms that you can have a visually identifiable structural abnormality, or you can also have some sort of electrical disturbance in terms of heart disease in broad terms?

WITNESS SKINNER: In broad terms, yes. One or the other in general, yes.

MORRIS SC: To that extent is it the case that with Long QTc, a certain percentage of patients can have a normal ECG?

WITNESS SKINNER: Indeed. If there's a familial Long QT syndrome, about a third of people in that family who are gene carriers will have a normal ECG on a single examination. Often if you repeat that examination, subsequent ECGs will be abnormal but that is certainly the case, yes.

MORRIS SC: Are there other things that can trigger arrhythmia other than just your genetic background?

WITNESS SKINNER: Yes.

MORRIS SC: Could you just--

WITNESS SKINNER: Lots of different things. One of course would be myocarditis. That was one of the issues in Laura's case. Medications, you know, given in toxic doses, and heart muscle diseases can cause heart rhythm disturbances.

MORRIS SC: What about infection?

WITNESS SKINNER: That's what I meant by myocarditis, so a direct infection of the heart can do this, and in Brugada syndrome, this rare condition, just a

fever can sometimes trigger a cardiac event, provided you are genetically susceptible.

MORRIS SC: What do you mean by genetically susceptible, Professor?

WITNESS SKINNER: So if you have Brugada syndrome and you have a gene in the sodium, cardiac sodium channel, SCN5A, and you are a child and you have a high fever, then you would be at risk of having cardiac arrest.

MORRIS SC: Are you aware whether there are any specific infective processes which have got a propensity to do this?

WITNESS SKINNER: In terms of the Brugada phenotype, any fever can do this. So if you're genetically susceptible, it can be a fever from cholestasis, you know, from gall bladder disease in an adult, or it can be pneumonia, or, or the flu.

MORRIS SC: Does anybody else wish to comment upon this evidence?

WITNESS COLLEY: No.

MORRIS SC: No? Professor Carola(as said), do you wish to comment on it?

WITNESS VINUESA: (No verbal reply)

MORRIS SC: No, okay, thank you. In relation to tab 47 that you were shown yesterday, on page 1 of that document we've got an incident history of back pain. Can back pain be triggered by some sort of cardiac event or stress?

WITNESS SKINNER: Well back pain is the great mimicker for everything, but I, I don't really think that a cardiac problem produces back pain. I would say it's more likely in this instance that back pain from various causes, it can lead to a vagal, a vasovagal type of event rather than the other way around.

MORRIS SC: When we talk about a vasovagal event, are we talking about a cardiac event or a--

WITNESS SKINNER: Sorry, a common faint is what I mean by that.

MORRIS SC: Unrelated to a cardiac cause?

WITNESS SKINNER: Correct.

MORRIS SC: Just on that issue, there was a document shown to you yesterday in which she presented with chest pain with tingling in the arms.

WITNESS SKINNER: Yes.

MORRIS SC: Do you recall that document you looked at yesterday?

WITNESS SKINNER: Yes.

MORRIS SC: I know we're looking at clinical presentation which may be capable of several interpretations, but can that be a sign of some sort of cardiac difficulty?

WITNESS SKINNER: Yes, of course. And I think that was presumably behind the presentation in May 2011 when she, she'd had a series of chest pains over the previous year, and I think although the doctor felt it was atypical, that would be the sort of thing where you'd be conscious of coronary artery disease and ischaemia. It's not the typical sort of presentation for a cardiac ion channelopathy like Long QT syndrome or something like this, but it's much more likely to be coronary artery disease. But I think the doctor was happy to exclude that on the tests that he did on that day.

MORRIS SC: You mean the ECG?

WITNESS SKINNER: The exercise test and the lack of ischaemia on the exercise test.

MORRIS SC: Professor Skinner, before lunch you were referring to a number of articles on a topic I can't recall, and my junior can't help me, but we might come back to that. You referred to a couple of articles. Just let me see if my notes--

WITNESS SKINNER: I think I can probably help you with that. Was it to do with the studies of sudden infant death and exome studies?

MORRIS SC: Yes.

WITNESS SKINNER: Yes.

MORRIS SC: What were the articles to which you referred and are you able to forward them to counsel assisting?

WITNESS SKINNER: Of course. I could - if the Court would like, I could prepare a brief summary. The main reason I was presenting that data was because there - although we have naturally enough focused on the potentially positive results from the genetic screen, I wanted to highlight that the - a large number of the jeans which are most commonly associated with this sort of scenario have not cropped up as having variants within them. So that was my main point.

MORRIS SC: I see. Just in relation to the - you've seen exhibit AF which is the report of Professor Vinuesa and Professor Cook? You've seen Professor Vinuesa's report have you?

WITNESS SKINNER: I have, yes.

MORRIS SC: If we go to page 14, do you have that diagram there? That's a

short summary.

WITNESS SKINNER: Yes.

MORRIS SC: To that extent have you heard of ADAMTS6?

WITNESS SKINNER: No.

MORRIS SC: We can see there that each of these four children have got ADAMTS6 in them, do you see that?

WITNESS SKINNER: Yes.

MORRIS SC: You wouldn't seek to postulate what that relates to would you?

WITNESS SKINNER: I would defer to my genetics colleagues. This is not a gene that I'm familiar with.

MORRIS SC: Let's deal with Professor Vinuesa, can you just tell us about the ADAMTS6 gene?

WITNESS VINUESA: Well we included this because this is a recent discovery. There was a publication in 2018, an Exome chip meta-analysis data, that identified novel loci as was stated with cardiac conduction and a single doubt in the title ADAMTS6, and they went on to perform some functional studies validating in vitro that there are common variants which one would not expect to be very deleterious, but even common variants led to impaired ADAMTS6 secretion and loss of functionalities in mice demonstrated a previously unappreciated role for ADAMTS6 in a particular function or the expression of a particular protein that is essential for myocardial conduction. So again very recent recent report, not very solid evidence, but this is the way we identify novel genes associated with cardiac conduction defects, so a lot to do but potentially a new lead into something that could be relevant.

MORRIS SC: Would anybody from the Sydney team wish to comment upon this?

WITNESS KIRK: Yes, look and I think this highlights the difference of approach. Professor Vinuesa is a very experienced and eminent researcher and is, I guess, approaching this in the way that you might approach a research project, thinking about possibilities, expanding the different, the different areas of knowledge that we currently have. Whereas our approach is more focused on known disease associations.

I'll just say in relation to this particular study, the printed - or one of the characteristics of that was that the subjects could only be included in the study if they did not have any known disorders of cardiac function. This was a study of variation in normal people, and the variants that were identified accounted for changes in the, in the duration of one of the elements of the ECG of .72 milliseconds. So, I mean I think I agree that it's an interesting gene that may at

some point prove to be relevant to human disease, but we're a long way from that point at present.

MORRIS SC: When we talk about human disease, we're talking about human cardiac disease?

WITNESS KIRK: Yes.

MORRIS SC: And we're talking about conduction?

WITNESS KIRK: Potentially.

MORRIS SC: You indicated the difference in approach between the research approach--

WITNESS KIRK: Yep.

MORRIS SC: --and the clinical approach.

WITNESS KIRK: Yes.

MORRIS SC: Because you as a clinician, and the three of you as clinicians, would essentially not action a clinical course to treat somebody with ADAMTS6 unless you saw pathogenicity--

WITNESS KIRK: Yeah.

MORRIS SC: --in the patients?

WITNESS KIRK: So we - I should say that we are all researchers as well, so clinician researchers, and so we might also if we saw something that we thought was a promising candidate initiate studies, but we'd have to confine ourselves in managing patients to what is known.

MORRIS SC: When you talk about what is known--

WITNESS KIRK: Yep.

MORRIS SC: --you're talking about what level of confidence?

WITNESS KIRK: Well for something like this, really you can't have any confidence. When we talk about managing patients you need a high level of confidence.

MORRIS SC: The ACMG guidelines are directed at clinical treatment rather than some sort of forensic investigation, do you agree?

WITNESS KIRK: Yes I think I'd agree with that.

MORRIS SC: Do we all agree with that?

WITNESS COLLEY: Yes.

WITNESS BUCKLEY: Yes, except that I think inherently the investigation of sudden death in children is a medical issue.

MORRIS SC: I don't disagree with that, but the primary focus of the ACMG guidelines is for a grading of satisfaction so that it can guide you in the advice that you give to patients, is that correct?

WITNESS BUCKLEY: Yes.

WITNESS COLLEY: Yes.

MORRIS SC: And the treatment options that may be efficacious?

WITNESS KIRK: Potentially, it depends on the clinical circumstance.

MORRIS SC: Now, Professor Skinner, do you agree with that?

WITNESS SKINNER: Yes. Yes, I do. I'm also a, a clinician researcher and I - I've been researching sudden death for the last 15, 20 years and I, I'm very interested in genes which might explain sudden death. That's all I would say, really. I applaud the move to generate hypotheses, but I guess the question is here, at what level in the current status of knowledge can we ascribe a reasonable level of possibility to something being a contributor to the disease?

MORRIS SC: I understand that, and thank you for that answer, but does not the emergence of the ADAMTS6 gene indicate the progression of genetic studies by researchers?

WITNESS SKINNER: Yes, it does.

MORRIS SC: And to that extent, you'd agree with me, Professor Skinner, that the fact is that the relationship between genes and genetic understanding and sudden death still has some considerable way to go. Do you agree with that?

WITNESS SKINNER: Yes, I expect so. There - we've come a long way, but we still only explain 15, 20% of, of our sudden unexplained deaths genetically. We don't know how many more are genetically, but we think we've probably - through studies that I alluded to earlier - found the main players through Whole Exome studies, but there may well be more.

MORRIS SC: Professor Vinuesa, do you have anything to add to this?

WITNESS VINUESA: No, but I - if, if I may say that, I mean, this is exactly what we do in our day to day activity, we take cases where we have a suspicion of - that a VUS may be pathogenic and we test it functionally. If I can just tell you a very brief example of one of our recent families that I think is relevant to this case. We recently were referred a family where there had been

four recurrent deaths in four consecutive children between the ages of 4 weeks and two months. This family had initially been referred to the most prominent laboratory in the United States, they had failed to reach a diagnosis.

When we received the DNA from the family, we reached a diagnosis within three months. We found two variants in the same gene that initially were thought to be a VUS. We established a collaboration with a, a specialist laboratory in the Netherlands that specialises in that gene. They were able to prove complete loss of function of this gene. Therefore, this variant was immediately reclassified as pathogenic, within three months. And the interesting thing about this case is that there were other children and other cases in the world that had been described with variance in this gene, but all of the deaths had occurred after ten years of age. This was the first case with four deaths where they all occurred in children below four months of age.

We were a little bit intrigued, thinking that there could be a second variant that could enhance pathogenicity and, since we have found another variant in the same gene that is X linked, we are testing it and another specialist is testing it. In the last year there's been a report that this gene causes pathogenicity in the same pathway. We are submitting this manuscript for publication. So, here we have a case where we have three variants in four children, the same variants. The probability that this occurs is 1 in 64,000. But, you know, we were surprised, but in a planet of 7 billion people, these extreme cases do occur.

So, again, this I think poses the question, are we trying to find something that is routine, ordinary, a common diagnosis, or are we also contemplating the probability that we might be dealing with something quite rare, unusual, with unusual type of genetics, that presents differently, earlier onset, than other variants in similar genes? And this is where, in our experience, we are seeing this all the time. We are reclassifying variants from VUS to pathogenic after validation in the laboratory and this is the beauty of genetics and why we are now going to probably increase, quite exponentially, the spectrum of variants that contribute to disease. Not just us, but the whole world is working at it.

MORRIS SC: And when you refer to this family and when we look at the ADAMTS6 gene, are these variants that are turning up in the population or within families?

WITNESS VINUESA: Look, there is a little bit of both. Some variants are initially identified by genome-wide association studies, so people only identify the common versions, the very frequent variants that have very small effects on the genes, and thereafter people include these genes in their candidate lists and can therefore focus their attention to rare variants.

Now, at the moment, you will be aware we have performed a very limited exercise by just going with what is known, with genes that are known to be candidates for sudden unexpected death. As new genes are discovered, we will expand our lists and some will be discovered by this time - this type of routine exercise of large population studies identifying loci that have some type

of function in cardiac abnormalities.

JUDICIAL OFFICER: Mr Morris, you're not asking for a five-year adjournment, are you?

MORRIS SC: Your Honour, certainly not, but the - I'll move on.

JUDICIAL OFFICER: Thank you.

MORRIS SC: Does anybody wish to comment on Professor Vinuesa's experience?

WITNESS KIRK: Well, just one thing about the ADAMTS6 variant is that it's present in one in 6,000 Europeans, so it's, it's rather too common to be a really plausible candidate. I was speaking more about the general approach. And we've done - we haven't had a similar family, but we've certainly done the same thing where we've contributed to the identification of new disease genes. So, it is true that that's an ongoing process.

MORRIS SC: That's what I wanted to ask you about. We've got laboratories around the world--

WITNESS KIRK: Yep.

MORRIS SC: --each of whom are acting independently, all right? And is it part of the ACMG guidelines that there be the provision and publication of new genetic relationships with disease association?

WITNESS KIRK: No, the ACMG guidelines specifically say that they're not intended for research application. It is good medical practice to pursue new potential findings and there is a very active international effort to do this. So, for example, there's a resource called GeneMatcher Exchange where, if you find something that you think may be relevant, you can put that up and then others who may have found something similar can get in touch with you, and we've participated in multiple gene identification exercises through that path. But that's not what the ACMG criteria are for.

MORRIS SC: Okay. But, Professor Buckley, you for instance have published a good number of papers on genetic associations with particular disease processes, haven't you?

WITNESS BUCKLEY: I've published a number, not so many. Could I come back perhaps to the point that Edwin was making about the frequency of this one variant, ADAMTS6? We, we have a frequency of sudden unexplained death in Australia of about one in 3,000, one in 3,300, that sort of order. This, this variant is present in the population at one in 6,000. You would assume, based on just those numbers, that 50% - if, if this was truly causative of disease, that 50% of sudden infant death would be due not only to ADAMTS6, but due to this one single mutation in ADAMTS6, and we don't see that.

MORRIS SC: Okay, well, just on that topic, it's fair to say isn't it - and I'll just go back and clarify the evidence that was given yesterday, that - and this is at T409, Dr Buckley, you indicated at line 6, and I'll read it to you, "As far as we can tell, we can identify somewhere in the range of 2 to 20% of causes - that is, of SIDS - can be attributed directly to monogenic genetic causes"?

WITNESS BUCKLEY: That's correct. Yeah.

MORRIS SC: Now, I just want to clarify this because, at page 410, Professor Kirk, you indicated, and I'll read it to you:

> "Just in regard to the assumptions, to say that this is a standard process that is routinely used and also in relation to your question about the information about 2 to 20% of SUDI not as having an identifiable genetic cause, that has the tendency to make our assumptions more conservative, because it effectively reduces that number of one in 3,300 to something much smaller than that"?

WITNESS KIRK: Yep.

MORRIS SC: Now, there seems to be an inconsistency between the two statements. Professor Buckley, you said that the range of genetic causes attributable to sudden infant death syndrome is between 2 and 20%?

WITNESS BUCKLEY: Yes, and I think that figures in that ballpark were echoed just a few moments ago by Professor Skinner.

MORRIS SC: Yes, okay. And, Professor Kirk, do you agree with that?

WITNESS KIRK: Yes, I don't think we're in disagreement.

WITNESS BUCKLEY: No.

MORRIS SC: It's just that you said that "2 to 20% of SUDI not as having an identifiable genetic cause"?

WITNESS KIRK: I must have misspoken, I'm sorry. What I'm - what I intended to say was that if we start with one in 3,000 and we say that 2 to 20% of those have an identifiable genetic cause, that means that you're really looking for up to one in, say, 15,000 - if I'm doing the numbers correctly - and we looked at variants that were as common as one in 1,000. So, the, the gap between those two numbers is wide and that's why it was a conservative assumption.

MORRIS SC: I understand. But in relation to an answer, Professor Buckley, you gave a moment ago when you talk about the percentage of sudden deaths in infancy and the relationship to ADAMTS6(as said), I'm not suggesting that ADAMTS6 is causative of 50% of SUDI deaths.

WITNESS BUCKLEY: Yeah, well, I'd, I'd agree.

MORRIS SC: The more interesting thing that I wanted to tease out was the fact that advances in genetics are continuing at pace. Do you agree with that?

WITNESS BUCKLEY: Yes, I would agree with that and they are being really driven, but I would come back to counsel assisting's comments yesterday, again that this is a multifaceted test where we're trying to correlate clinical presentation with genetic findings in a laboratory and that the two are essential components. One of the difficulties with this family is that the presenting clinical features, Alison Colley summed up very beautifully as being normally developing, normal grown children who, without any preceding significant medical illnesses, presented with a sudden catastrophic event. So, we are, in essence, trying to tie normality to variants in genes. That's a very difficult proposition. Alison, am I misquoting you?

WITNESS COLLEY: No, I, I think that's very reasonable. It is interesting, you know, trying to find new genes and looking for novel new genes, and I think it is, obviously as a clinician, very important that we look very carefully at the phenotype and marry the phenotype with the genotype together. With the ADAMTS6, remember that the four children have this very common variant but it is not seen in the mother, and so we're assuming that this has come from Mr Folbigg, the father and, as far as we're aware, he's healthy and well in his fifties, without any cardiac events that would be of the type that would indicate a conduction defect, as far as I'm aware.

MORRIS SC: And it's the case that that extended family, or not having access to that extended family on the Folbigg side, necessarily affects the capacity to segregate the genetic information. Do you agree with that?

WITNESS COLLEY: I do. If we had a pathogenic variant that we felt tied in with a phenotype, I think it would be vitally important to be able to segregate it, but we actually don't have that here. So, segregating something that's one in 6,000 people is not something that I think is useful.

MORRIS SC: Okay. Professor Vinuesa, do you agree with that?

WITNESS VINUESA: Look, I would like to make a comment. I think we are only contemplating the most simple scenario of the single gene causing disease. There is increasing evidence of digenic causes of disease. We've dealt with many, you probably have as well. When we have digenic causes, two genes coming together, first, the frequency of each of those doesn't need to be so ultra-rare, so we can cope with frequencies like the one we've just talked about for this ADAMTS6. Furthermore, there is good evidence that even common variants can substantially modify the incidence of disease, and if I may quote one, "Crotti et al have provided evidence that the common polymorphism KCNH2 (30% carrier amongst whites - 30% carrier frequency) may modify the clinical expression of latent LQT2 mutation."

So we can have either different genes, two that, rare variance, one common and one rare, many common. If you look at the pedigree we have in our

screens we have quite a lot of variance that could be coming together to cause part of this disease, and then in those cases the frequency changes, we just can't say that because the frequency is not that rare we have to exclude a variant. On the other hand, coming back to your point, yes, and we still have to remember they show of non-penetrants. I mean, an unaffected parent does not exclude it because, as we say, most of these cardiac conditions it is typical to find non-penetrance carriers of pathogenic mutations and we have seen them where the trees have both come to MHY6, et cetera.

MORRIS SC: Does anybody have anything to say about Professor Vinuesa's statement about the combination of genes or is that a matter that's generally accepted?

WITNESS KIRK: Well, I would say that one of the problems with that is that we could conduct this exercise with any family with four children and come up with a very similar looking list of genes, and so the problem really comes down to interpretation and we have no way to interpret that kind of information that is meaningful.

MORRIS SC: Given the current state of knowledge--

WITNESS KIRK: Yes.

MORRIS SC: --of the interaction between genes?

WITNESS KIRK: Yes, well, yeah, that, that's true. We're a long way from being able to interpret that kind of information usefully.

MORRIS SC: And that is a matter which is the subject of research, isn't it?

WITNESS KIRK: Yes.

MORRIS SC: Professor Colley, in relation to pathogenicity, do you make that comment given that in the family tree which has been prepared there's a "? SIDS death" in one of Craig's eight siblings?

WITNESS COLLEY: The "? SIDS death" is in one of Craig's siblings children. So that's a cousin, it's a second degree relative there to Craig. So but it, it, it is interesting and it's something that would have been good to know some more information about, but when I spoke personally with Craig, as I mentioned yesterday, he really didn't have any more information. He wasn't sure that it was a SIDS at all. He didn't know what the cause of that death was or, in fact, when exactly that baby died, at what age, I mean, that baby died or in what circumstances.

If we postulate and assume that that baby had what the four children of Kathleen and Craig had and think about how a genetic condition might be inherited in a family that would give those cousins both an affected status, we can certainly rule out X linkage and we rule out mitochondrial condition. If we said it's possible that there'd be a dominant, an autosomal dominant condition

then we're postulating again that, non-penetrance, with two possibly three - so also Craig and Michael's, one of their parents, three people who lived to middle age with no features of the phenotype at all, yet now five children with a very severe phenotype at a young age. That's certainly a lot to postulate. So autosomal dominant and deceased penetrance with that many people in the family is not likely but, of course, possible.

The other option is, of course, an autosomal recessive condition where, you know, it takes two faulty genes for the phenotype. It's reasonable that, if Craig had one copy and Michael would have one copy, that's a 50% risk, but it does mean that Kathleen and Michael's partner, the mother of that baby who ? SIDS would also have to have a copy of the faulty gene and in this sort of family where you have non-consanguineous white Caucasian northern European family, it would be highly unlikely. In the area where I work in southwest Sydney were 50% of my population are blood relatives and usually multiply so, it's the sort of thing I do see quite commonly, but in my white Caucasian Australian non-European population it would be highly unlikely, but not impossible.

MORRIS SC: Professor Vinuesa, you held your hand up.

WITNESS VINUESA: I would like to comment. There is another possibility which is that they have a second gene that is different from Kathleen and that is not unheard of. There are families that co-inherit, you know, BRCA1 and BRCA2 mutations and we know that some of these pathways there are up to ten or 20 proteins that come together. So it is not unconceivable that the second mutation that comes to compound the effect is a different mutation in the wife of Michael and in Kathleen. So a digenic inheritance would be compatible with this presentation.

MORRIS SC: Do you agree?

WITNESS COLLEY: Possible.

MORRIS SC: If there was any other sudden infant death in the Folbigg line in, let's say, another generation, would that add to the picture?

WITNESS KIRK: It's hard to speculate about that without a full pedigree, knowledge of the circumstances. There's a whole lot of missing information that makes that too speculative to really meaningfully respond to, I think.

MORRIS SC: Okay.

WITNESS BUCKLEY: I think also that individual, of course, would not only have a Folbigg family member as one parents, they would also have a different person from a different lineage.

MORRIS SC: I'm working on the basis that we're not dealing with a consanguineous family--

WITNESS BUCKLEY: Sure.

MORRIS SC: --but is the assessment of another generation of any use in the segregation of genetic cause?

FURNESS SC: Your Honour, we have what we have. Your Honour is dealing today with the information that's available. If your Honour wishes for evidence to be sought and given in relation to what might happen tomorrow or what might happen in the family that we don't know about, I leave it in your Honour's hands.

MORRIS SC: It's just that one question about segregation, whether it assists in segregation, your Honour.

JUDICIAL OFFICER: Okay, I'll allow you one answer.

MORRIS SC: Thank you, your Honour.

WITNESS KIRK: If you've got a clear clinical condition and a variant as a plausible explanation for that condition, then segregation can be useful, and if you have multiple individuals who are affected in a family, as I said in my earlier evidence, then tracking a variant through a family can provide useful evidence for or against the possibility that it is pathogenic.

MORRIS SC: Thank you. Professor Vinuesa?

WITNESS VINUESA: I would agree that the more information, the better.

MORRIS SC: Yes, okay.

JUDICIAL OFFICER: Mr Morris, do you have any further questions of Professor Skinner?

MORRIS SC: I've got a couple and I'm going to move onto it now. Professor Skinner. Can you hear me?

WITNESS SKINNER: Yes, I can.

MORRIS SC: I now recall the literature. We were talking about Calmodulin and the studies that you were referring to that deaths from Calmodulin are associated with the patient being awake; do you recall that evidence?

WITNESS SKINNER: Yes.

MORRIS SC: Would you be able to provide us with literature references to those studies, please?

WITNESS SKINNER: Yes, I believe some of those were actually put forward by your - by Professor Vinuesa's team but we can provide some more of those--

MORRIS SC: Thank you.

WITNESS SKINNER: --certainly, yeah.

MORRIS SC: Do you have any comment to make about the last couple of minutes of questioning of the other experts?

WITNESS SKINNER: Only the general one, really, and, and that is we, we could come back here in ten years and have this same conversation. I think - this is really up for the Court to decide but we can speculate forever about what might be and what might happen and what experiments in mice might mean for the human being. Right now all we can look at what we know now or what we have reasonable confidence in knowing now and I, I think we're going to end up in, in a circular conversation unless we agree what the endpoint is here. I, I think the ideas that are put forward by Professor Vinuesa's team are great. It's, it's, it's a good thing to think laterally and to think wisely, multigene inheritance and so on, but at this stage we just don't have enough information about that to make meaningful judgments in, with the current knowledge of phenotype genotype data.

MORRIS SC: So there's more work to do, Professor?

WITNESS SKINNER: There's always more work to do. The question is, for me, what does this Court want us to do now with the information we have at the moment?

MORRIS SC: There's another issue, Professor, and that is that some evidence was given yesterday about the future development of genetic studies and whether we have a robust understanding now of what genetics can tell us about cardiac arrhythmia and other heart conditions and other causes of sudden death, and you'll recall those questions and answers yesterday, I take it?

WITNESS SKINNER: What do you specifically refer to?

MORRIS SC: How about I do it this way? One of the articles to which reference has been made is an article by Lam, Exome Sequencing identifies a novel mutation in the MYH6 gene in a family with early-onset sinus node dysfunction, ventricular arrhythmias and cardiac arrest. Do you recall that article?

WITNESS SKINNER: Yes.

MORRIS SC: That was Lam and another co-author was Christopher Semsarian.

WITNESS SKINNER: Yes.

MORRIS SC: This is an article which is predicated on the basis that there

remains - and I'll take you to the introduction at the bottom of the first column - "However, there remains a group of individuals in whom the cause of sudden cardiac death is unknown;" do you see that?

WITNESS SKINNER: Yes.

MORRIS SC: And there's a footnote there, footnote 2, "Semsarian and Hamilton, The key role of the molecular autopsy in sudden unexpected death?"

WITNESS SKINNER: Yes.

MORRIS SC: Are you familiar with that article?

WITNESS SKINNER: That was a - I, I don't know it in detail. That was a review article written in 2012 some time before we did the three year all core study of sudden death including in molecular autopsy across Australia and New Zealand. So it's a bit old but, yes, I remember it vaguely.

MORRIS SC: Yes, but that's the sort of article which has driven further study, correct?

WITNESS SKINNER: Yes, I suppose. It's, well, it's a review of - actually, that was actually trying to get people to use the molecular autopsy in a clinical sense from research which has already happened rather than promoting further research, I believe.

MORRIS SC: If we go to the second column there, the observations made are that "There are some cases which have got no identifiable mutation in known disease-causing genes and, therefore, highlight the necessity to discover new susceptibility genes for inherited arrhythmogenic disorders."

WITNESS SKINNER: Absolutely.

MORRIS SC: That remains the case now; do you agree?

WITNESS SKINNER: Absolutely, yes, I do.

MORRIS SC: Do you agree that there are advances in next generation sequencing platforms and targeted DNA capture strategies which hopefully will allow vastly greater capacity to sequence many thousands of genes at one, offering the ability to identify new genetic loci and mechanisms for cardiac electric abnormalities; do you agree with that?

WITNESS SKINNER: Yes, I do, to a degree. I think Whole Exome Sequencing is, is allowing us to study a, a large number of genes but it's going to be a long time before the human phenotype information catches up with that. We're at the stage now where we're finding more and more and more genes as we've seen in this Court, where actual, the clinical implications are just not known and the job of people like me and, and Professor Vinuesa and other clinicians in the room is to try to tie that information together. So this is

going to be an avalanche of information, an avalanche of genes that hitherto have been hidden from us, you're quite right, but it's going to take a long time before we catch that up to the human phenotype.

MORRIS SC: I understand. This was a study involving a three generation, the assessment of a three generation Australian family?

WITNESS SKINNER: Yes.

MORRIS SC: The final conclusion, if you go to page 144, above the heading "Conclusion" it says in the last five lines:

> "Overall the current available data suggests that MYH6 anomalies can cause cardiac conduction abnormalities, but given the limited pedigree size and enigmatic disease characteristics of this family, the role of MYH6 variants in the electrical system of the heart warrants further functional analysis."

Do you agree with that?

WITNESS SKINNER: Yes, I think what he's saying is that yes, we need further work and this paper is inconclusive of itself because it's so small.

MORRIS SC: But the fact is that in terms of genetic studies events within single families can contribute to the understanding of genetic cause of disease, do you agree with that?

WITNESS SKINNER: Yes, when it's put together with other information, indeed yes, careful study of entire pedigrees, yes.

MORRIS SC: It's fair to say Professor Skinner that there is a, if I can use a lay expression, that - well really tying in with something Professor Carola stated was that it's thought that the interplay between two separate genes may increase the potency of any genetic anomaly. That's a theory that's about but not well understood. Do you agree with that general postulate?

WITNESS SKINNER: I do, and I think Professor Vinuesa has a good point and she quoted a particular example of a common genetic change which modifies the severity of disease, and we certainly know there are several examples where common variants can modify the severity of disease, and we will learn more about that with time for sure.

MORRIS SC: Professor Skinner, this Inquiry has heard evidence from some SIDS experts, namely Professor Rosemary Horne, that with respect to sudden infant death, up to or just under half of victims of sudden infant death have had mild viral infection in the weeks beforehand. That's a statistic which she quoted to us. Is that anything that you've come across in your studies?

WITNESS SKINNER: I wouldn't dispute that. Infants, as we heard earlier on, repeatedly have upper respiratory tract infections. It's a normal and repeated

phenomenon and it wouldn't surprise you to find that a child that had died with one had an infection, if it's routine, to get about eight infections a year, then we're bound to find some of that, yes. And I guess one of the questions that logically would arise from that is did the virus somehow trigger some sort of cardiac event? In our field we've been looking for that, that evidence, and the only evidence really to date that we've found is related to the cardiac sodium channel gene I referred to earlier and it's linked to Brugada syndrome and the fever. However, that tends to really be older children, but I am quite sure that that could happen in the infant as well, high fever and triggering a cardiac event in somebody with Brugada syndrome.

MORRIS SC: What about the, I might as well ask you as we've got you here, the postulate about a pro-inflammatory cytokine IL-6 which can introduce a level of toxicity which can trigger an arrhythmia. Have you engaged in any research on that sort of area?

WITNESS SKINNER: No, I don't have any knowledge of that area at all, cytokines. It's not an area that I can speak to.

MORRIS SC: To that extent would you defer to an infectious diseases expert or immunologist?

WITNESS SKINNER: Yes I would. I will say that of course as a cardiologist I do see rhythm problems related to myocarditis, but I can't speak to the cellular and inflammatory mechanisms involved in that.

MORRIS SC: I think there was some evidence given earlier that sudden infant death is unlikely to have been caused by a genetic variation in the CALM2 gene.

WITNESS SKINNER: I wouldn't say it's unlikely. I would say that it's not proven yet. I mean, there's been lots of studies of sudden death, including from my own institution and I can provide you with the data that I was quoting earlier on from the Mayo Clinic UK study, and this is a Whole Exome study of some 400 infants, comparing Whole Exome Sequencing against a control population, and CALM didn't come out of that one as potentially interesting but four cardiac genes did, and that was SCN5A, RYR2, KCNH2 and KCNQ1, and it's only the variant in KCNQ1 which piqued my interest because of that.

MORRIS SC: If you could provide us with those articles at some point Professor.

WITNESS SKINNER: Certainly.

MORRIS SC: Professor Vinuesa, just in relation to your recent case study of four deaths in the one family, was that a cardiac related gene?

WITNESS VINUESA: No, it was an immune related gene.

MORRIS SC: An immune related gene, okay. Was that related at all to

cardiac function? Was it postulated or not?

WITNESS VINUESA: Not really. What would be your opinion Todor?

WITNESS ARSOV: We don't know.

MORRIS SC: Counsel assisting asked the four witnesses yesterday about the knowledge today about genetic understanding and what's going to happen tomorrow. You gave your answers. In the light of today's evidence would you wish to revise that evidence you gave yesterday?

WITNESS BUCKLEY: Could you read us specifically what the question that counsel assisting put to us please?

MORRIS SC: Okay. Counsel assisting asked this question:

> "We'll come back to the array results. Can I ask you whether or not the fact that we know what's happening today but we don't know what's going to happen tomorrow affects the reliability of the work you've done today in the light of the fact that your science is rapidly progressing."

Dr Colley said:

> "I think the likelihood even in a decade's time that we would find something startlingly different is low because of the Whole Genome Sequencing techniques that have been used and the quality of the data that we've been told about. Now in saying that there is clearly going to be new technology and new interpretation, but at this stage looking to the future as much as we can, I'm not envisaging that we're going to have to redo all this in a different way.
>
> FURNESS SC: With a different result perhaps.
>
> WITNESS COLLEY: With a different result, I can't, I don't believe that's going to be the case but it's a changing field."

Counsel assisting, "Is the field changing so much that it's pointless to express an opinion today?" Answer Colley:

> "No, I don't think so, because we did go for Whole Genome Sequencing. Now our genomes aren't going to change that much, I don't think. I mean, there is natural selection but I don't think we're going to see a change in the genome and we've done the test hypothesis-free to interrogate the genome as much as we can."

That was the general flavour of the evidence, and I just want to understand whether perhaps I'm at a cross-purpose with you. So I'd like some clarification. Is what you are saying is that you are confident that the raw data that was produced by your study, you're reasonably confident about the, if I can call it

the recovery rate, and that you think that that baseline information, the raw results, have been pretty well done, and that the chance that there is not going to be - you're confident that that will remain useful information into the future. Is that what you're saying?

WITNESS BUCKLEY: Yes I think so, given that we have, we've got very limited material here. It's very unlikely that we're going to be able to retest all of these samples using a putative technology that comes along in another five years. I think the data we have are reliable. I think the very fact that our Canberra colleagues and ourselves analysed these data, using different approaches, similar, using different models, but very largely we came up with a very similar set of variants that we thought were plausible, that we were confident in, that we thought should be considered as part of this matter. I, I don't see that, that we're going to come up with a very substantially different view into the future unless there is some radical change in sequencing technologies in the next few years. We have what we have. These are the data that we are best able to explain.

They seem to be consistent between two groups by and large and where we depart is where - it's the different weighting and interpretation that we put on those, and I think to a degree some of the analysis reflects, says more about ourselves perhaps than about the data, that it reflects our different views. I think together the data presented by Professor Vinuesa, the data presented by us, are a remarkable snapshot of the genetics of this family at this time which we are trying to understand in the light of current knowledge.

MORRIS SC: Professor Vinuesa, do you agree with that general comment by Dr Buckley?

WITNESS VINUESA: I agree that in terms of technology, we will probably not come up with a substantial number of different variants, but we are only analysing 1% of the genome, we have not even considered 99% of the non-coding mutations. We know that - we have agreed that 50% of genetic conditions cannot be diagnosed today - of monogenic genetic conditions, and the expectation is that as soon as we have better tools to explore the significance of structural variants, other missense mutations in enhancers or cryptic supplies in sites throughout the genome might give us a, a whole new list of variants to look at.

Also, we are limited by current knowledge of genes and their function. We still don't understand how at least one third of the genes in the genome work or what their function is, so I expect that over the next few years there will be more genes that will have been implicated in cardiac disease, there will be more variants. So, I think the interpretation can significantly change in a few years, not the raw data. I agree with you, the technology will not change, the raw data will not change, but we will make better sense of it in a few years.

JUDICIAL OFFICER: Could I just ask Dr Buckley a question? The testing that you did, did it destroy the samples?

WITNESS BUCKLEY: Yes. It - there are - there are some remaining samples. There are some tissues from Patrick, there are some fibroblasts that remain. There is - we were careful with some of the blood spots, that there is some material remaining on each of those, so - but every, every crank of the handle does consume a reasonable amount of the available material.

JUDICIAL OFFICER: So, if I gave Mr Morris his five-year adjournment, when we resumed in five years' time we might not be able to get all the material that we need?

WITNESS BUCKLEY: There is a possibility, your Honour, that that is the case. I, I couldn't put a - I will be honest, and I could not tell you what the probability is, but it's possible, certainly.

JUDICIAL OFFICER: Yes.

WITNESS BUCKLEY: Not the least of which, it's another five years in which deterioration can occur. We've already seen that some of these blood spot cards, which will be probably the main resource into the future, have been extensively contaminated with bacteria--

JUDICIAL OFFICER: Yes, thank you.

WITNESS BUCKLEY: --and so there is an ongoing degradation process as well.

JUDICIAL OFFICER: So, I refuse your five-year adjournment, Mr Morris.

MORRIS SC: May it please the Court. This is just to clarify what was being spoken about yesterday, we expect the DNA recovery is reasonably robust, but the thing that is happening at pace is the publication of new research discoveries relating to the genetic link between a particular phenotype and the genetic discovery, is that right?

WITNESS BUCKLEY: Yes, that's proceeding at a - it's, it's a - it's a golden age of genetics.

MORRIS SC: I'm sorry?

WITNESS BUCKLEY: It's a golden age of genetics, it really is.

MORRIS SC: I mean, it really is--

WITNESS BUCKLEY: The, the question is not whether discovery is going to proceed at pace, it's the, the area where that's happening best are in the monogenic disorders, people with very clear inherited or de novo causes where the - we're striking unusual and rare phenotypes. I'm not sure that this family - apart from the fact there has been a striking catastrophic event in all of these, the children were remarkably normal, at least to an experienced geneticist's eye, until that event occurred.

MORRIS SC: Professor Vinuesa, do you have anything to add to Professor Buckley's observations?

WITNESS VINUESA: I mean, if, if we are considering sudden unexpected death as the first manifestation of some of these cardiac diseases, which has been published, I don't think there is a reason to exclude these children because of apparently normal phenotypes, or exclude the possibility of a cardiac event, particularly because we don't have ECGs from the children and sometimes the only manifestation is the cardiac arrhythmia, sometimes transient, it sometimes can only be detected with 24-hour monitoring. So, the assumption we made for this exercise is that there might be a sudden cardiac event precipitated by an arrhythmia, and we just don't have the information, and this often occur(as said) in apparently otherwise healthy children.

WITNESS BUCKLEY: But we see no compelling evidence of a variant that might explain it under current knowledge in the data that you and I have in front of us for these four children.

WITNESS VINUESA: Well, I mean, there's where our differences in interpretation could lie.

MORRIS SC: Now, Professor Skinner, I think the prospective study that you referred to in your evidence, which was published in the New England Journal of Medicine, I think that was 2016?

WITNESS SKINNER: Correct.

MORRIS SC: And, in terms of the results, you identified 490 cases of sudden cardiac death?

WITNESS SKINNER: Yes, I--

MORRIS SC: Have you got the paper in front of you?

WITNESS SKINNER: I don't, but I, I - I'm happy for you to proceed and if I don't recall an aspect, I'll tell you.

MORRIS SC: Okay. I'll just run through the results, which is published on the first page. That was a study from persons one to 35 years of age?

WITNESS SKINNER: Yes.

MORRIS SC: And, specifically, persons under one year of age was excluded?

WITNESS SKINNER: Correct.

MORRIS SC: To that extent, within that cohort that you were studying, in 40% of cases the predominant finding was unexplained cardiac death; is that correct?

WITNESS SKINNER: Correct. Correct.

MORRIS SC: The study indicated that younger age and death at night were independently associated with unexplained sudden cardiac death as compared with explained sudden cardiac death?

WITNESS SKINNER: That's right.

MORRIS SC: And a clinical relevant cardiac gene mutation was identified in 31 out of 113 cases, 27%, of unexplained sudden cardiac death in which genetic testing was performed?

WITNESS SKINNER: Correct.

MORRIS SC: Are we to draw from that that genetic testing was performed in 113 cases of the 419?

WITNESS SKINNER: That's right. I, I don't have the full details in front of me but the, the, the actual method of testing was quite varied across the cohort 'cause it matured during the time of that study, so that we started with a gene panel and ended up with whole exome but, yes, broadly speaking I think you're right.

MORRIS SC: During follow up a clinical diagnosis of an inherited cardiovascular disease was identified in 13% of the families in which an unexplained sudden cardiac death occurred; does that--

WITNESS SKINNER: Yes. Yes, that, the, the, the problem with the study from that perspective is that this was over three years and screening families often takes many years, but to that point that it was published that's the case, yes.

MORRIS SC: In up to one-third of cases of sudden cardiac death amongst children and young adults, bearing in mind the cohort we're talking about aged one to 35, a cause of death was not found after a comprehensive autopsy examination that included toxicologic and histologic studies?

WITNESS SKINNER: Correct.

MORRIS SC: Unexplained sudden cardiac death is often attributed to cardiac arrhythmia caused by a cardiac iron channel dysfunction which is undetectable on a conventional autopsy?

WITNESS SKINNER: Yes.

MORRIS SC: Non-cardiac conditions may also cause sudden death which is clinically indistinguishable from sudden cardiac death; do you agree?

WITNESS SKINNER: Yes, I think in that study there was around a 6% finding

of potentially informative variance in the epilepsy genes, some sudden unexplained death in epilepsy genes.

MORRIS SC: Yes, and I was going to go on. In the article it says, "For example, patients with epilepsy have a higher rate of sudden death than persons without epilepsy and sudden unexpected death in epilepsy is the most common cause of death relating to epilepsy?"

WITNESS SKINNER: Yes.

MORRIS SC: That's the paper to which you were referring yesterday, I think?

WITNESS SKINNER: That's right.

MORRIS SC: Okay, thank you. Professor Buckley, we were talking a little earlier, and Professor Colley, about these children appearing to be in good health, right?

WITNESS COLLEY: Right.

WITNESS BUCKLEY: Yes.

MORRIS SC: To that extent, do you accept the observations made by Professor Skinner that infection and fever can cause cardiac dysfunction or would you defer to an infectious diseases specialist or immunologist?

WITNESS BUCKLEY: I, as I recall, Professor Skinner was saying there was good evidence in the context of SCN5A variants that hyperpyrexia could cause cardiac dysfunction. Am I misquoting you, Professor?

WITNESS SKINNER: No, you're - that, that's exactly right. It would be a trigger for a, an event in somebody who's genetically predisposed--

WITNESS BUCKLEY: I think--

WITNESS SKINNER: --not a direct--

WITNESS BUCKLEY: I think both Professor Vinuesa and myself looked very hard to find SCN5A variants in this family and we - I certainly didn't see any. Carola, can you perhaps comment? I--

WITNESS VINUESA: No, we didn't although it does illustrate that these type of phenomenons happen and it could happen with other channelopathies, presumably.

WITNESS BUCKLEY: So, so, yes, I accept the comment by Professor Skinner that SCN5A people - people with SCN5A pathogenic variants are susceptible to cardiac dysfunction when they have a high, a high temperature. I'm not sure that that has any relevance to the family that we are looking at here because none of the children, as far as either group have been able to define, do have

those variants.

MORRIS SC: What about immunology issues?

WITNESS BUCKLEY: Sorry, I think I'd have to defer to an immunologist for--

MORRIS SC: Okay.

WITNESS BUCKLEY: --for that, I think.

MORRIS SC: Thank you. With respect to the issue of the inherited disorders such as Hunter Syndrome and so forth, Professor Colley, you made observation yesterday about the lack of dysmorphia in the parents?

WITNESS COLLEY: (No verbal reply)

MORRIS SC: You'll have to say yes or no.

WITNESS COLLEY: Sorry, yes. Yes, when I saw the parents in 1991 is the only time I saw them, I think, or maybe early 92, that was the last time I saw them.

MORRIS SC: With respect to the photographs of Patrick specifically, and I'm directing these questions to Patrick, is it the case that you saw any sign of dysmorphia in Patrick in the photographs?

WITNESS COLLEY: As you know I didn't see him personally. He was--

MORRIS SC: No, I understand.

WITNESS COLLEY: --already deceased, but in the photographs, no, I didn't. We did hear yesterday though that this is a progressive condition and there can be changes in a gene that hasn't caused the cells to change and the substances to be accumulated in the cells to change the phenotype at such a young age, and it is that changed cellular phenotype which actually can lead to the death. So, although I didn't see any physical features or dysmorphic features that would allow me to make a diagnosis of Hunter Syndrome, that's a separate independent issue to the actual genotype but we do know that if you don't have the phenotype at that particular age you're unlikely to have the sudden death because of the condition.

MORRIS SC: But it remains possible that you can have a sudden death, even without the dysmorphia having set in place, do you agree?

WITNESS COLLEY: I think I'd defer to our metabolic specialist, but my answer to that would be highly unlikely but I'm going to defer to the metabolic specialist, Dr Kirk.

WITNESS KIRK: So the thing to understand about the mucopolysaccharidoses is that they are multisystem conditions, they are not

just a heart condition, they affect many parts of the body, heart disease as Professor Skinner has said is a late feature of this condition and we would expect that in someone with Hunter who had developed heart disease, that they would have had signs in other body systems, so I think - I do think I agree that the photograph certainly by itself does not exclude the diagnosis of Hunter Syndrome in a child that age, definitely not.

MORRIS SC: And the secret there is the child of that age, do you agree?

WITNESS KIRK: Yeah if it were an eight year old then different.

MORRIS SC: Because it's fair to say that patients with Hunter Syndrome generally appear normal at birth, do you agree with that?

WITNESS KIRK: Yes, they may have hernias but otherwise, they don't have to have those.

MORRIS SC: Professor Colley you nodded?

WITNESS COLLEY: Agree.

MORRIS SC: And that the even with patients with the severe phenotype, the clinical signs and symptoms usually emerge between two and four years of age, do you agree with that?

WITNESS KIRK: Yes in retrospect, so they're often recognised at those ages, in retrospect you can often see subtle features in photographs.

WITNESS COLLEY: I would agree, sometimes you see subtle features that you think, this is a mucopolysaccharidosis even though you're not perhaps sure exactly which one but you're thinking this is a metabolic condition or an inborn error of metabolism and then you might start investigations to try and find out which one.

MORRIS SC: And it's fair to say isn't it that the available of genetic testing since 2003 has made that far more - made that diagnostic process far easier do you agree?

WITNESS KIRK: In general in genetic conditions yes, not for Hunter Syndrome, that was already well understood in 2003.

MORRIS SC: Some of the signs and symptoms of Hunter Syndrome include of course facial features, that's the--

WITNESS COLLEY: Yes, the mucopolysaccharidosis, the inborn error of metabolism in a lot of these conditions it is a non-specific finding but quite a consistent finding, that you would get coarsening of facial features, and I think coarsening is an important word, because all babies look a bit different, some babies are going to look more coarse at birth than other babies depending on their parents' features and their ethnic background, so we would say it's

important to see how the child has changed over time and the features have coarsened.

MORRIS SC: And other issues that you might end up with is cognitive and developmental delay?

WITNESS COLLEY: Yes.

MORRIS SC: Reference is made to spinal cord compression?

WITNESS COLLEY: Not one that I've seen.

WITNESS KIRK: Yes, so in relation to cognitive delay there are essentially two groups, so there are some people who do not have that and some who do, and spinal cord compression is a consequence of abnormal formation of the spinal bones and also of accumulation of abnormal material in the tissue surrounding the spinal cord.

MORRIS SC: With Patrick it's probably I mean, carpal tunnel syndrome is something that would be very difficult to identify in an infant I would've thought?

WITNESS KIRK: It also wouldn't occur in an infant, it's a later feature.

MORRIS SC: It's a late onset okay. What about and also issues of hyperactivity, aggression and impulsivity in an infant of Patrick's age?

WITNESS KIRK: You couldn't assess that.

MORRIS SC: You couldn't assess that. But another feature which can be associated with Hunter Syndrome is seizures, is that correct?

WITNESS KIRK: That's a relatively late consequence of the condition as well and it relates to progressive deposition of material in the brain.

MORRIS SC: Is it in an aggressive variant or subtype of Hunter Syndrome, can it trigger seizures at an early age?

WITNESS KIRK: I would think not in the first year but it can trigger seizures at an early age yes.

MORRIS SC: Professor Vinuesa, do you have anything to add to any of this or is this something that's really outside your field?

WITNESS VINUESA: It is outside my field but we have provided a couple of reviews to the Inquiry.

MORRIS SC: Also hearing loss can be a feature?

WITNESS KIRK: Very common problem.

MORRIS SC: And that can happen early, is that correct?

WITNESS KIRK: Yes it relates to blockage of the Eustachian tube that drains the middle ear and accumulation of fluid in the middle ear and because that's a small structure it's vulnerable to small changes.

MORRIS SC: And I think following on from that, one of the features is also recurrent ear infections but I think that's really--

WITNESS KIRK: It's all connected.

MORRIS SC: It's connected?

WITNESS KIRK: Yes, and one makes the other worse.

MORRIS SC: What about persistent rhinorrhoea?

WITNESS KIRK: Yeah it's a real problem.

MORRIS SC: Just tell me about that?

WITNESS KIRK: So they have, so rhinorrhoea means a runny nose and in Hunter Syndrome it's quite a prominent feature and it's often quite thick and problematic.

MORRIS SC: Professor Colley?

WITNESS COLLEY: I agree.

MORRIS SC: And frequent respiratory infections?

WITNESS KIRK: Yes that's something that's progressive over time and relates to gradual changes in the shape of the chest and also deposition of material in the airways, so that children become increasingly vulnerable, partly it's got to do with the thick mucus and also to changes in the airways that lead to increasing frequency and also severity of chest infections.

MORRIS SC: So we're talking about, you were talking about the gluggy nature of the rhinorrhoea?

WITNESS KIRK: Yeah.

MORRIS SC: We're talking about mucosal production?

WITNESS KIRK: Yes.

MORRIS SC: Which then gives rise to difficulties with ongoing respiratory infections later in life?

WITNESS KIRK: The issues are connected.

MORRIS SC: And it can also lead to respiratory obstruction?

WITNESS KIRK: That's a later feature, but well so one of the earlier manifestations of that is obstructive sleep apnoea, which is a common problem in all children with all mucopolysaccharidoses, perhaps a bit less in type 3 but it is a common issue.

MORRIS SC: And we're talking about noisy breathing and snoring?

WITNESS KIRK: Well it's more than just that, they certainly have that but if a child has obstructive sleep apnoea then they might have periods of stopping breathing during the night.

MORRIS SC: Now hepatosplenomegaly?

WITNESS KIRK: Yep.

MORRIS SC: We're talking about enlargement of the--

WITNESS KIRK: The liver and the spleen yeah, that may not be obvious in an eight month old, although at post-mortem you probably would expect to see signs of that so that you'd expect to see that and to see changes in the tissues.

MORRIS SC: We talked about umbilical hernia and inguinal hernias?

WITNESS KIRK: Yes.

MORRIS SC: Which was absent from this--

WITNESS KIRK: Yes but that I mean doesn't tell you a lot, not every child has that feature.

MORRIS SC: It's non-specific is that what you're saying?

WITNESS KIRK: It's non-specific in that many things can cause that and it's also not a sensitive feature in the sense that not every child with Hunter Syndrome has that.

MORRIS SC: Diarrhoea can be a common feature?

WITNESS KIRK: Yes, yes it can, type - MPS III where that's really prominent but yes.

MORRIS SC: MPS III?

WITNESS KIRK: Different disorder.

MORRIS SC: That's a different disorder?

WITNESS KIRK: Different disorder.

MORRIS SC: So what would - if I can just zap through the last few matters, we're - dysostosis, what's that?

WITNESS KIRK: I'm not sure.

MORRIS SC: D-Y-S-O-S-T-O-S-I-S multiplex?

WITNESS KIRK: Oh okay, so one of the major areas where the condition affects is cartilage and bone development, so when you do X-rays on children with mucopolysaccharidosis, even fairly early on but it's a progressive feature, there is an abnormality of the bones, particularly in the joints, because of problems with the cartilage but also the formation of the bone itself and multiplex means it's widespread throughout the body.

MORRIS SC: We're also dealing with, also features of it are growth retardation and claw hands?

WITNESS KIRK: They're often actually big to start with, but yes down the track the growth can be restricted, claw hands is meaning that there is restriction, again it relates to the connective tissues in the body, so the cartilage would come broadly under that category I suppose but the connect tissues around the joints and in particular in the hands are affected so there is restriction of movement.

MORRIS SC: Are those features of this disorder which come on a bit later on?

WITNESS KIRK: All of these are progressive changes that gradually get worse over a period of years, apart from the hernias which as I say can often be present at birth.

MORRIS SC: And that's the ordinary course of the development of the disease is it?

WITNESS KIRK: Well it's a pretty consistent course of development yeah.

MORRIS SC: And we've talked about cardiac valve disease?

WITNESS KIRK: Again a progressive relative - well I think there can be valvular changes, I would defer to Professor Skinner on this but it is part of the progression of the condition.

MORRIS SC: Professor Skinner, Professor Kirk has put you back into this debate, the valvular changes, are they something that occur over time with Hunter Syndrome or are they something which can present fairly early on?

WITNESS SKINNER: I haven't seen them present early, if that's helpful, I think they're quite - they're quite subtle early on, they wouldn't cause a

functional abnormality certainly in infancy and pre-school but they develop later on.

MORRIS SC: In relation to - and we've also got the possibility of visual disturbance, is that right, in Hunter?

WITNESS BUCKLEY: Yes, although it's not as prominent in some of the other mucopolysaccharidoses, so there are some where there are clouding of the cornea, you can get mild clouding of the cornea in Hunter but it's not nearly as prominent in other forms of mucopolysaccharidoses, well as in type I, Hurler Syndrome.

MORRIS SC: Now in relation to mucopolysaccharidosis?

WITNESS BUCKLEY: Yep.

MORRIS SC: What are the other common variants?

WITNESS BUCKLEY: Okay, so type I is a spectrum from a condition called Hurler Syndrome, which is quite similar to Hunter Syndrome, severe early onset, many of the same features that we've talked about, but that can depending on the deficiency of the enzyme, present more mildly, through to a condition which causes severe joint disease in a person who can live to adulthood and have normal intelligence usually, most people with type I have severe intellectual deficits.

MORRIS SC: In relation to type I is it the fact that genetic testing has excluded the type I in this case?

WITNESS BUCKLEY: We didn't see any variants in the gene for any of the other mucopolysaccharidoses and also the screening that was done is general to all of the different known mucopolysaccharidoses.

MORRIS SC: Professor Vinuesa, in relation to the - do you agree with Professor Kirk about the fact that the, apart from Hunter Syndrome, the other mucopolysaccharidosis conditions did not present on your genetic testing?

WITNESS VINUESA: Yes I agree, may I make a comment about the molecular diagnosis.

MORRIS SC: Yes.

WITNESS VINUESA: The value. I mean in these gene reviews that we cited in our response, reference 6 of our response it is said that identification of a hemizygous IDS pathogenic variant by molecular genetic testing confirms the diagnosis of MPS II in a male proband and may be useful in persons with an unusual phenotype or a phenotype that does not match the results of GAG analysis, with these I think there's some suggestion of molecular and genetic diagnosis could be valuable in cases that are not so straightforward, perhaps in unusually young age of presentation et cetera, but I defer to the metabolic

experts, we are just are citing from a review that we cited in our response.

MORRIS SC: Do you have anything to say about that literature to which--

WITNESS KIRK: If you had a case that was presenting early you'd be expecting that to be a child who was exceptionally severely affected and you'd expect the testing to be if anything the urine testing if anything, more abnormal than with a later case. I think where you might conceivably consider this would be if you had a very mild condition and you were suspecting that it might be and I think it's still unlikely, very unlikely the urine would miss that but perhaps you might go to genetic testing then.

MORRIS SC: Was that available--

WITNESS KIRK: Yes.

MORRIS SC: --as at 1991, that other genetic testing?

WITNESS KIRK: 91, no I don't think so. I, I'd have to take that on notice.

MORRIS SC: Professor Colley, you were there?

WITNESS COLLEY: I'll have to - may I just say it's Dr Colley, not Professor.

MORRIS SC: Sorry, Doctor, I'm sorry.

WITNESS COLLEY: As you might have learnt that when I make a clinical diagnosis of an inborn error of metabolism like a mucopolysaccharidoses, I'd be quick to hand over to the metabolic experts. So I think what we have discussed yesterday was that we felt that the studies that were done on Patrick, both for urine metabolic scree and in the post mortem, didn't give us any positive thinking that Patrick had clinical features of a mucopolysaccharidoses, and in particular Hunter's.

MORRIS SC: Can I just split that up.

WITNESS COLLEY: Yep.

MORRIS SC: When you talk about clinical features, the urine tests, are you saying that he urine test was a clinical feature which you thought was not really consistent with mucopolysaccharidoses?

WITNESS COLLEY: No, the clinical features were the fact that this child didn't have some of those phenotypic features that you and Professor Kirk had just gone through. For example the clawed hands and the blocked Eustachian tubes and the spinal changes. So - no I think they're separate, they're the clinical features, whereas the urine test is an investigation and is a separate issue, but what Professor Kirk is saying is that when you have children who have those clinical features you would expect, because the clinical features are caused by them having an abnormal gene which is producing an abnormal

protein, you would expect that the investigation, the urine metabolic screen, would be able to detect that.

MORRIS SC: Okay. Can you just explain to me this urine metabolic screen and what you're looking for in the urine metabolic screen?

WITNESS COLLEY: I'm going to defer that to Professor Kirk because he's actually worked in the laboratory that does those tests.

MORRIS SC: Right.

WITNESS KIRK: Are you referring specifically to the mucopolysaccharidoses testing or the test more broadly?

MORRIS SC: The mucopolysaccharidoses testing.

WITNESS KIRK: So what you're looking for is, as I discussed yesterday, there are two tests that were conducted. One is quantitative, so looking for the total amount of mucopolysaccharide material--

MORRIS SC: I'm sorry, I just missed that. You're looking for the total?

WITNESS KIRK: The total amount of mucopolysaccharide material that is present in the urine sample relative to the creatinine as a measure of concentration of the urine. And then the qualitative, or semi-qualitative test which gives you information both about amount, but more importantly the pattern of any glycosaminoglycans that are present in the urine. And there are distinctive patterns that indicate the presence of one of these conditions, and to some degree go to distinguishing them. But, for example, type I and type II have quite similar patterns on that second test. So in that situation - say you saw a child in who you expected the diagnosis, you would do the urine testing, which you would expect to be strongly abnormal, and then you may have to go to enzymatic testing to distinguish which of those it is, and then for various reasons, for example testing of other family members to see if they are carriers, you may well then go on to genetic tests, so that's the usual sequence that we would follow.

MORRIS SC: And that's the sequence you'd follow today?

WITNESS KIRK: Yes, with the possible exception that we might go straight to genetic testing if we were confident of the situation, skipping the enzymatic testing these days.

MORRIS SC: Would that be based on looking at the clinical history of the child and also the urine test?

WITNESS KIRK: Yeah, it'd be a combination of features. Usually the urine test has been done for a specific reason. I have seen a child with mucopolysaccharidoses type I in whom test was done for an unrelated reason, and this was essentially an incidental finding, and it was only later on that the

features became apparent.

MORRIS SC: That must have been a very young child?

WITNESS KIRK: Yes.

MORRIS SC: These levels in the urine that we're looking for, does that have any variation over time of day or diurnal variation or--

WITNESS KIRK: The absolute levels certainly vary with the concentration of the urine. That would be the major, the major variation, and that's why we - the laboratory normalises against creatinine, but there may be slight variations from day to day and from time to time. Generally speaking people who have Hunter's Syndrome excrete a lot of mucopolysaccharide material and so that variation is not really a problem in terms of making the diagnosis.

MORRIS SC: In terms of the excretion of this material, is there a variation from the point at which the child is born to say 12 months, or is that not known?

WITNESS KIRK: I don't know the answer to that. I can tell you that the - however, I can tell you that the test is more likely to come back as a false positive in the early month or so of life because we quite often have children who have urine metabolic screens which includes a lot of things, including this test, for other reasons and we get a positive, a mildly raised level, and, and when we follow that up it has normalised. So I suspect the answer is that there is a bit more in the early month or two of life, and that it reduces somewhat. But I don't know that for certain.

MORRIS SC: That's okay. To that extent you mentioned dilution.

WITNESS KIRK: Yes.

MORRIS SC: Is that a question of fluid input and output?

WITNESS KIRK: Exactly that.

MORRIS SC: In terms of accuracy to determinant, between the time taken between the sample, the sample being taken, and it being processed, is that an important consideration, and if so why?

WITNESS KIRK: I'm not completely certain of the answer for this assay. The, the usual practice when we're doing this type of testing is to freeze the sample fairly quickly and transport it, but that mainly relates to some other analytes. So I can't completely answer that question with certainty.

MORRIS SC: Is it possible that that can give rise to a false negative?

WITNESS KIRK: It's conceivable. I can tell you that since the New South Wales Biochemical Genetics Service introduced this test in 1997,

we've had no known false negatives for any of the mucopolysaccharidoses. That probably ought to only count up until a few years ago, because you could imagine if we'd missed one two or three years ago the diagnosis might not yet have been made. But even so, there are a lot of these tests done. A number of diagnoses have been made over that time and we're not aware of any false negatives. And because of the varied way in which samples are collected and sent, my impression would be that it's unlikely to influence it but I can't completely exclude the possibility.

MORRIS SC: Okay. I want to ask you, until such time as there is the development of symptoms and so forth, would you agree that Hunter Syndrome type II can be difficult to clinically diagnose?

WITNESS KIRK: Absolutely.

MORRIS SC: Because the symptoms to which it can lead can mimic a lot of common childhood illnesses and features?

WITNESS KIRK: Early on, yes.

MORRIS SC: Is it fair to say, just going over this matter, that I think is it called a urinary GAG test, or a GAG test?

WITNESS KIRK: Yes.

MORRIS SC: That it does not necessarily rule out the diagnosis of a Hunter Syndrome or a mucopolysaccharidoses disorder?

WITNESS KIRK: Well if you - so I don't know if you've got the report in front of you - the wording on the report says that this essentially excludes all known mucopolysaccharidoses, and I would regard that as being accurate. There is no test in existence that is 100% perfect.

MORRIS SC: Would you agree with the proposition that false negative results may occur due to dilute sample?

WITNESS KIRK: Yes, that's why we normalise against creatinine.

MORRIS SC: And variations in GAG excretion over time?

WITNESS KIRK: It'll - so that will depend on the condition as to how much of a problem that is. Most important for MPS IIIB.

MORRIS SC: And overlap in ranges between affected and unaffected patients?

WITNESS KIRK: That really should be a problem for the Hunter Syndrome first test.

MORRIS SC: Is it fair to say that GAG tests, and I'm talking about a paper by

Ian Young Lee called "Isolation identification and quantification of urinary glycosaminoglycans".

WITNESS KIRK: Glycosaminoglycans, but GAGs will cover it.

MORRIS SC: Are we talking about the same--

WITNESS KIRK: Yes.

MORRIS SC: That was a study that was published in 2003 which observed that GAG turnover should ensure that control groups are precisely matched for age?

WITNESS KIRK: I'm sorry, I'm not familiar with that study and I'm not sure what, what that refers to.

MORRIS SC: Perhaps I might give you a copy of it overnight.

WITNESS KIRK: Sure.

MORRIS SC: I just want to run this--

WITNESS KIRK: But I may be able to answer that, which is that for all tests in children we need to have reference ranges that are specific to the child's age.

MORRIS SC: Okay, right. Do we know what the reference range is for a child under 12 months?

WITNESS KIRK: Yes, that information should be available, yep.

MORRIS SC: Is it fair to say that the levels of GAG are low in childhood and reaches a peak in the years ten to 19?

WITNESS KIRK: Look I couldn’t comment on that, I don't know the answer.

MORRIS SC: Why don't we do this. I'll give you a copy of this paper.

WITNESS KIRK: Yep.

MORRIS SC: Overnight, and perhaps you - Dr Colley if you want to inform yourself - and perhaps we might just consider the reference ranges that are appropriate for consideration. Was that a suitable course on this topic?

JUDICIAL OFFICER: Yes, certainly if you need it, yes.

FURNESS SC: Perhaps we might be provided with a copy.

MORRIS SC: Yes, certainly.

JUDICIAL OFFICER: Have you finished with Professor Skinner?

MORRIS SC: Just excuse me a moment. He may be released from service.

JUDICIAL OFFICER: Professor Skinner, thank you very much. We appreciate it very much taking the time out to help us through what we all think is a fairly difficult and complicated Inquiry, but your assistance has been significant, thank you.

WITNESS SKINNER: Thank you very much. It's been an honour and pleasure to be involved. Thank you.

WITNESS SKINNER WITHDREW

AUDIO VISUAL LINK CONCLUDED AT 3.58PM

MORRIS SC: I'm about to head into a course of cross-examination which will require consideration of certain clinical records and so forth, and there is no way that I'm going to finish that this afternoon. But subject to discussions with my learned friends I think that will be the extent of the further discussion I will have with these experts who have kindly given their time. So I would expect that I would be about an hour with that, maybe a little longer, but I'll certainly try and narrow it down overnight if I can so as not to put these good experts to any further inconvenience.

JUDICIAL OFFICER: Thank you. Ms Furness, tomorrow Professor Fahey is coming?

FURNESS SC: And Professor Ryan, and Professor Ryan can only be here tomorrow as can Dr Buckley. I don't anticipate that Professor Ryan and Professor Fahey will be very long from my point of view.

JUDICIAL OFFICER: So your anticipation for tomorrow's proceedings is that they should conclude?

FURNESS SC: Subject to my friend, yes.

JUDICIAL OFFICER: Conclude at 4 o'clock are you talking about or are you concluding at lunchtime?

FURNESS SC: I would conclude at lunchtime but I suspect it will be closer to 4 o'clock because it’s not me alone.

JUDICIAL OFFICER: We'll adjourn for today and resume tomorrow morning.

<THE WITNESSES WITHDREW

ADJOURNED PART HEARD TO WEDNESDAY 17 APRIL 2019