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TRANSCRIPT OF PROCEEDINGS

INQUIRY INTO THE CONVICTIONS OF KATHLEEN MEGAN FOLBIGG

MONDAY, 15 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

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SPECIAL INQUIRY

THE HONOURABLE REGINALD BLANCH AM QC

MONDAY 15 APRIL 2019

INQUIRY INTO THE CONVICTIONS OF KATHLEEN MEGAN FOLBIGG

PART HEARD

JUDICIAL OFFICER: Yes, Ms Furness.

FURNESS SC: Thank you. I appear to assist your Honour with my learned junior Ms McGee, instructed by Ms Richards from the Crown Solicitor's Office.

MORRIS SC: I appear with my learned juniors Mr Cavanagh and Ms Reed in the interests of Ms Folbigg.

JUDICIAL OFFICER: Thank you Mr Morris. Yes?

FURNESS SC: Thank you. On the last occasion, which was the directions hearing held on 1 April which followed a detailed letter, your Honour will recall, from those assisting Ms Folbigg in relation to foreshadowed reports. It was foreshadowed on that occasion that there may or would be a psychiatrist's report by the end of that week, as well as a further report from the cardiologist, Professor Waddell-Smith, who had provided a report and a report from a metabolic expert. Since that time those assisting your Honour have been informed that there will be no report from the metabolic expert and has had no word in respect of the other two. Further, on 12 April, which I think was Friday, a report from a molecular geneticist was received by the Inquiry, which had not been foreshadowed either in the directions hearing or in any other manner.

In addition to those matters, those assisting Ms Folbigg have organised for her to have a consultation with a cardiologist on 18 April and again, those assisting your Honour have done what they can do to ensure that the results, that is the raw results of that consultation, be made available immediately to Professor Skinner and anyone else with a legitimate interest in receiving it in order to make a report. After this week, the Inquiry will be sitting in the week of 29 April in order to hear evidence from Ms Folbigg and that will be the completion of the evidence. So I draw those matters to your Honour's attention.

JUDICIAL OFFICER: Thank you. Mr Morris, we need to have some fairly firm orders made about reports. There are a couple of reports that have been brought in very late.

MORRIS SC: Yes, I understand, your Honour.

JUDICIAL OFFICER: The simple situation is that because we've had a lot of experts in this case and we've had trouble getting court facilities and getting all the experts here to give their evidence, it's not really open to have further reports that can't be answered by experts who have already been and gone. That's simply not an appropriate way for the Inquiry to run. So that the situation simply has to be that apart from the tests that are being done on Ms Folbigg and the results of that, and it's very unfortunate that that's had to be delayed for as long as it has, but apart from that there really has to be an absolute end to reports by the time we finish sitting this week. Are you envisaging any reports?

MORRIS SC: We've still got the psychiatrist's report, we're still waiting on that, your Honour. We haven't received that yet. With respect to the electrophysiology report, the testing of Ms Folbigg, your Honour will recall that we were having difficulties with Department of Corrective Services trying to facilitate that.

JUDICIAL OFFICER: I think the real problem has been that the doctor that you have briefed to do it is away and won't be back from overseas.

MORRIS SC: I don't wish to cavil with that issue, but the problem was that the Department of Corrective Services were telling us they required two weeks and we had an earlier date available when the doctor could have performed that electrophysiological testing, but it didn't fit within their two week timeframe.

JUDICIAL OFFICER: I understand.

MORRIS SC: It was with the intervention of counsel assisting that they were able to modify that two week timeframe, but in the intervening period we had already made the appropriate arrangements and the doctor had made his arrangements to travel overseas. So, your Honour, it's not as if we have not been attempting to get this testing done at the earliest available opportunity, but we were simply constrained by requirements that were being imposed upon us by the Department of Corrective Services. It's regrettable and we accept that, but by the time that counsel assisting have managed to facilitate an earlier time, the doctor had made his arrangements and we couldn't do anything with it.

JUDICIAL OFFICER: Okay.

MORRIS SC: But on the broad issue, I understand your Honour's concerns. Your Honour would appreciate that we are dealing with very, very complex issues of medicine that this timetable was established late last year and we were aiming to work towards it, but your Honour would appreciate that through the forensic process, so far as Ms Folbigg's legal team is concerned, we make an enquiry of one specialist, who then identifies another issue that we need to deal with which results in a further enquiry. To that extent, your Honour, I can understand the commission's great concern to resolve this and I've always said that we will try and meet the timetable, but in order to present Ms Folbigg's case clearly, we have had to deal with a great number of experts and the

metabolic expert that counsel assisting raised with you has disengaged because she doesn't have the time to deal with it. The fact is, we're now stuck with a corner of the evidence that we can't properly deal with--

JUDICIAL OFFICER: Sorry, when you say that, you're making it sound as though this is an adversarial procedure, it isn't.

MORRIS SC: No, I understand that, your Honour, but it has been a feature and I say this without any criticism at all, that areas which Ms Folbigg has presented to this Inquiry for consideration, areas of science, have not otherwise been advanced before this Inquiry. To that extent, I refer to the immunology and infectious diseases material of Professor Blackwell and Professor Clancy. That will, in our respectful submission, form a very important part of the evidential landscape which your Honour has to take into account and so to that extent, while I accept that it's not an adversarial system, the areas of science that have been presented to your Honour for consideration has been at both ends of this bar table. To that extent, we have been trying to identify gaps in the evidence and try and meet it in a very short timeframe.

JUDICIAL OFFICER: The Inquiry has been as well, and it's not a short timeframe, it began in August last year and it's not going to go on until August next year and it's as simple as that. It has to come to an end. Are there any other loose ends that you are even contemplating at this stage?

MORRIS SC: That metabolic expert, I would still wish the opportunity to consider consulting with somebody and your Honour, I understand that this Inquiry was advanced in August, the direction was given in August. Your Honour will be well aware that it wasn't until 4 December that we were granted legal aid funding.

JUDICIAL OFFICER: Let me just ask Ms Furness about the metabolic expert. Ms Furness?

FURNESS SC: In relation to a metabolic expert, Professor Kirk, who is sitting in the witness box now, was the head of metabolic services at one stage in New South Wales and I'm sure that he would be happy to talk with my friend about metabolic matters. Indeed, Professor Kirk works in the same institution as Dr Ellaway and therefore would be perfectly appropriate for my friend to speak to with respect to metabolic matters. Can I then, just while I'm on my feet, turn to molecular matters. Dr Buckley is a molecular geneticist. So these areas of expertise are available to your Honour and should my friend wish to discuss matters with them, I'm sure they'd be happy to do so.

JUDICIAL OFFICER: All right, thank you.

FURNESS SC: My friend has mentioned Professor Waddell-Smith, who had provided a report and it was foreshadowed she would provide a further report and that has not been dealt with, so perhaps your Honour might also hear about--

MORRIS SC: We don't have a further report from Professor Waddell-Smith.

JUDICIAL OFFICER: Thank you. Mr Morris, as I said, this is not an adversarial procedure. We have the experts in the metabolic area and we're not going to delay while you go off in the hope of contradicting whatever they're going to say. They may say something that's in your favour, I don't know and when they're not called here on the basis of give an adverse report in respect of your client, they're called here as experts. They will give their evidence as experts. You have, as counsel assisting says, the opportunity of speaking to them and I would not be prepared to adjourn any further evidence taking in respect of that area, when we have experts here who are giving what I hope is completely impartial evidence about that area of the Inquiry. So far as the Inquiry is concerned, those experts will be enough. Are there any other experts that you're even contemplating?

MORRIS SC: No, your Honour.

JUDICIAL OFFICER: Thank you, then I can indicate now that the curtain will definitely come down on Wednesday in respect of the evidence, apart from any evidence that might come out of the testing of Ms Folbigg, which can't be done until the 18th.

MORRIS SC: I understand.

JUDICIAL OFFICER: So far as the end of the expert evidence otherwise finishing by Wednesday, if you do come up with some other report that needs investigation, then we will have to deal with that. But otherwise, Wednesday is the end of the expert evidence.

MORRIS SC: I understand, thank you, your Honour.

JUDICIAL OFFICER: Thank you.

FURNESS SC: In relation to the report of the molecular geneticist that was received on Friday, I'm instructed that that expert received material that was potentially in breach of your Honour's non-publication order in that the genetic material was limited to those who had been effectively approved by those assisting your Honour and that person was completely unknown to those assisting your Honour, until the report was received on Friday. I don't know whether my friend wishes to address that?

JUDICIAL OFFICER: Is the report by a Dr McDonald?

FURNESS SC: It is, your Honour.

MORRIS SC: I'll have to make some enquiries.

JUDICIAL OFFICER: All right, thank you.

FURNESS SC: One final matter, if I may? Your Honour has made a number of non-publication directions in relation to the matters to be dealt with in these hearings. Firstly, a direction was made on 11 February restricting publication of the genetic sequencing data and information resulting from the interpretation of that data and any report given to the Inquiry about that information and that I asked your Honour to vary that direction to permit publication of the reports tendered into evidence and the oral evidence given about those reports.

JUDICIAL OFFICER: Yes, well I vary the order to permit publication of both the reports tendered into evidence in the Inquiry about the information and the oral evidence given about those reports.

FURNESS SC: Thank you, your Honour and secondly, a non-publication direction was made on 6 March restricting publication of health records of Ms Folbigg produced to the Inquiry by Justice Health and any report given to the Inquiry about those health records. Again, your Honour, I ask that the direction be varied to permit publication of the records and the reports about those records tendered into evidence and oral evidence given about those reports.

JUDICIAL OFFICER: I make an order varying the order to permit publication of the records and the reports about those records tendered into evidence in the Inquiry and the oral evidence given about those reports.

FURNESS SC: Thank you, your Honour and I note that some of the health records of Ms Folbigg have been redacted to permit only relevant records to be available.

JUDICIAL OFFICER: Yes, thank you.

FURNESS SC: This week the Inquiry will primarily hear evidence about advances in the field of genetics since Ms Folbigg's trial in 2003, and the application of those advances to the understanding of the deaths of the four Folbigg children. As I outlined in the first opening address, some genetic related investigations had been undertaken in respect of the children by the time of the 2003 trial. The results of those investigations were described as normal and did not indicate the need for further testing or investigation.

Significant advances have been made in the field of genetics since the trial. Those advances permit a much broader scope of investigation than was possible in 2003. Genomic sequencing technologies emerged in 2009. Since 2013 two major genomic sequencing technologies have become mainstream, Whole Exome Sequencing sequences the whole exome which is that small part of the genome, approximately 1% to 2% of the whole, that is involved in coding for proteins. Proteins are the key components of cells and damage to them can cause serious, if not catastrophic problems.

This part of the genome is the location of the majority of variants that cause developmental or cognitive disabilities and disorders. Whole Genome Sequencing sequences all of the genome that is accessible. In addition to the

exome, this comprises non-coding elements in the genome and mitochondrial DNA. This technology enables hypothesis free study of DNA where a known or presumed diagnosis as a starting point is not needed. Rather, DNA sequences are studied and variants are interrogated against the known healthy human genome, and the phenotype or clinical features of a person.

In 2015 the American College of Medical Genetics and Genomics published standards and guidelines for the interpretation of sequence variants, including assessing the pathogenicity of the variants. The ACMG standards refer to variants being pathogenic, that is causative of disease, likely pathogenic of uncertain significance, likely benign and benign. This terminology has been employed in the reports prepared for and to assist the Inquiry.

Material produced to the Inquiry by the New South Wales Ministry of Health in compliance with summonses your Honour issued included samples containing DNA from each of the four children, blood spots taken from each of the children at the time of their birth as part of the Newborn Screening Program and held at the Children's Hospital Westmead were available. In respect of each of Patrick, Sarah and Laura, tissue samples taken at the time of their autopsies in 1991, 1993 and 1999, and fixed in glass and wax block slides held at the Coroner's Court were also available.

In respect of Patrick, additionally available were kidney, liver, skin, skeletal muscle and heart tissue samples taken at the time of his autopsy in 1991, and frozen at minus 80 degrees. In respect of Sarah, additionally available was one tube of extracted genomic DNA from fibroblasts and two ampules of archived fibroblast cells stored in liquid nitrogen held also at the Children's Hospital at Westmead. In respect of Laura, additionally held at the Children's Hospital Westmead was formalin-immersed brain tissue taken at the time of her autopsy in 1999.

In December 2018 the Inquiry was informed that Ms Folbigg had provided to her legal representatives a sample for the purpose of genetic testing. Ms Folbigg consented to the sample being made available to the Inquiry for further testing.

The interpretation of genetic data involves consideration of both the genetic pathology and the clinical presentation of a person. It is a single but multi-faceted interpretation process. Accordingly, the Inquiry gathered together a multidisciplinary panel of experts to interpret and provide opinions about the data produced by the genetic testing undertaken for the Inquiry, and the available clinical information in respect of each of the children and Ms Folbigg. These experts are associated with two separate laboratories with genetic sequencing interpretation capabilities, in Sydney and in Canberra.

Dr Michael Buckley is a genetic pathologist and Clinical Director of the New South Wales Health South Eastern Area Laboratory Services at the Prince of Wales Hospital in Sydney. He holds a PhD in the field of molecular genetics obtained in 1991. Professor Edwin Kirk is a genetic pathologist and clinical geneticist at the New South Wales Health South Eastern Area

Laboratory Services, as well as co-head of the Centre for Clinical Genetics at the Sydney Children's Hospital. He has additionally trained in paediatrics and provides a cardiac genetics clinical service which focuses on adults and children with cardiomyopathies and disorders of cardiac rhythm, and as I indicated earlier, Professor Kirk was the head of metabolic services at a New South Wales facility.

Dr Alison Colley is a clinical geneticist and the Director of Clinical Genetics Services for various local health districts in New South Wales. She has trained in paediatrics as well as clinical genetics. She is a conjoint senior lecturer at the University of New South Wales, and Dr Colley is a renowned dysmorphologist and she will explain what that means.

Professor John Skinner who is with us by AVL is a paediatric cardiologist and cardiac electrophysiologist working as a consultant at Starship Children's Hospital in Auckland, New Zealand. He is an Honorary Professor in Paediatrics Child and Youth Health at the University of Auckland.

Professor Matthew Cook is a Professor of Medicine at the Australian National University and a practising clinical immunologist at Canberra Hospital. He is a co-director of the Centre for Personalised Immunology at the Australian National University and medical director of the Canberra Clinical Genomics Laboratory. That laboratory is accredited to conduct bioinformatics analysis of DNA and RNA sequences such as those produced by Whole Exome Sequencing and Whole Genome Sequencing.

Professor Carola Vinuesa is an Australian National Health and Medical Research Council Principal Research Fellow and Professor of Immunology at the Australian National University. She is also the chief scientist at the Canberra Clinical Genomics Laboratory, of which Professor Cook is the medical director. Together with Professor Cook she is also the co-director of the Centre for Personalised Immunology. Professors Cook and Vinuesa were assisted by Dr Todor Arsov, a visiting fellow at the Centre for Personalised Immunology. He holds PhD in Biomedical Sciences and Masters of Genetic Counselling. Each of those, with the exception of Professor Cook, will be giving evidence.

The Inquiry held three consultation meetings at which the interpretation panel experts discussed the options for genetic testing on the produced samples. On the basis of these discussions, Whole Genome Sequencing was conducted on DNA extracted from a frozen liver tissue sample from Patrick, DNA in the existing sample extracted from fibroblast from Sarah. DNA extracted from a blood spot sample from Caleb, and DNA extracted from the sample from Ms Folbigg.

Whole Exome Sequencing was conducted on DNA extracted from a blood spot sample from Laura, which was unsuitable for Whole Genome Sequencing because of microbial contamination of the sample. The Australian Genome Research Facility conducted the sequencing on the samples of Sarah, Patrick and Ms Folbigg, and the Victorian Clinical Genetics Service conducted the

sequencing on the samples of Caleb and Laura. All of those matters were agreed by the panel.

At the New South Wales Health Pathology Services Genetics Laboratory at the Prince of Wales Hospital in Sydney variant analysis of the sequencing data was conducted through a genomic analysis bioinformatics pipeline called Genomic Annotation and Interpretation Application, GAIA. At the Canberra Clinical Genomics Laboratory variant analysis of the sequencing data was conducted through a separate bioinformatics pipeline known as the Sydney laboratory and the Canberra laboratory for simplicity.

Ultimately each laboratory analysed the same data and the same genes. Almost 1,400 unique candidate genes were identified for analysis. In addition, the data was reanalysed, considering firstly cardiac, non-cardiac genes which had been published in relation to sudden death in infancy or childhood. Secondly, genes associated with childhood neurological disorders. Third, genes associated with immunology. Four, genes associated with metabolics, and finally likely pathogenicity in any phenotype not restricted to sudden death in infancy or childhood. It was also agreed by the expert panel that the ACMG standards and guidelines would be used for assessing the pathogenicity of variants.

All experts involved in the interpretation of the sequencing data were provided with documents relative to, and relevant to, the phenotype or clinical presentation of the children and Ms Folbigg, and your Honour has heard a deal of evidence about that. In short, the phenotype or observable clinical features of the children is of healthy, well-grown, normally developing children who are normal in appearance, each of whom suffer a catastrophic event, leading to death instantly in three of them and severe neurological sequelae in the fourth child which precedes his late death.

The relevant medical history and results of historical and other recent cardiac related investigations of Ms Folbigg have been considered by the experts as part of the interpretation process. Further information will be available from a testing schedule to be conducted on Ms Folbigg on 18 April as indicated earlier. Dr Buckley, Dr Colley and Professor Kirk prepared a joint report interpreting the significance of genetic variants identified through the Sydney pipeline present in the children and in Ms Folbigg and potentially relevant to the children's causes of death “The Sydney report”.

Professor Cook and Professor Vinuesa with the assistance of Dr Arsov prepared a joint report and a supplementary report interpreting the significance of genetic variants identified through the Canberra pipeline “The Canberra report”. The Canberra report concluded that no known pathogenic or likely pathogenic variants in genes that could explain unexpected death were found in four out of four of the children. The Sydney report came to the same conclusion and added that none of the variants identified were deemed causal for the phenotype in the children. The key difference of opinion expressed in each of those reports is as to three variants, primarily relating to cardiac function. One variant was only found in Patrick, another in Sarah and Laura,

and the third was found only in Laura and Caleb. Your Honour will be hearing a deal of evidence about these three variants.

Professor Jon Skinner prepared a report specifically addressing cardiac related variants in the children's and Ms Folbigg's genes as reported by the Sydney and Canberra pipelines, and the cardiac clinical presentation of each of them. He concluded that the available clinical phenotype data and genetic analysis in respect of the children and Ms Folbigg provide no convincing evidence for the presence of any known form of cardiac inherited disease as a potential cause for the sudden death of the four children.

Professor Monique Ryan is a senior paediatric neurologist and Director of the Department of Neurology at the Royal Children's Hospital in Melbourne. She was engaged by those representing Ms Folbigg to report on her assessment of Patrick's neurological condition in respect of the ALTE primarily. She concluded that she was not convinced that Patrick's clinical history was consistent with him having neurological deficits resulting from a single hypoxic-ischaemic episode on 18 October 1990, which has been referred to as his ALTE. She listed a number of alternative diagnoses which she said were potentially causative of his neurologic condition, which she said could be the subject of Whole Genome Sequencing. Her report dated 15 March 2019 was prepared without knowledge of the sequencing results in respect of Patrick.

Associate Professor Fahey, a paediatric neurologist and clinical geneticist, is Head of Paediatric Neurology at the Victorian Paediatric Rehabilitation Service at Monash Children's' Hospital. He prepared a report, at the request of the Inquiry, following the Whole Genome Sequencing in respect of Patrick. He was provided with Professor Ryan's report. To assist his analysis, Professor Fahey provided Dr Buckley with a list of 204 genes associated with childhood neurological disorders for analysis. Now, most of those genes had been identified and analysed, however the data was separately reanalysed.

After receiving those results, Professor Fahey concluded that the testing at the time of Patrick's acute or apparent life-threatening event and death, and the genomic testing conducted at the request of the Inquiry, have excluded any recognised conditions associated with genetic epilepsies, encephalopathy, cardiac arrhythmias, or sudden death, including the alternative potential diagnoses identified by Professor Ryan. So, Professor Fahey looked at the sequencing done in respect of those alternative diagnoses provided by Professor Ryan. He opined that the comprehensive investigations virtually eliminated a recognised genomic cause for Patrick's presentation. Professor Fahey and Professor Ryan will give evidence on Wednesday in respect of these matters, your Honour.

Thank you, your Honour. I call Professor Kirk, Dr Buckley, Dr Colley--

JUDICIAL OFFICER: We need to swear the witnesses.

AUDIO VISUAL LINK COMMENCED AT 10.33AM

LTS:DAT

<ALISON FIONA COLLEY AND MICHAEL FRANCIS BUCKLEY, SWORN, EDWIN PHILLIP ENFIELD KIRK AND JONATHAN ROBERT SKINNER, AFFIRMED (10.33AM)

FURNESS SC: I'll start with you, Professor Skinner. Will you tell the Inquiry your full name and address, your work address?

WITNESS SKINNER: Yes, Jonathan Robert Skinner. I work in the Paediatric Cardiac Services at Starship Children's Hospital in Auckland.

FURNESS SC: And your qualifications, Professor?

WITNESS SKINNER: MBChB, Bachelor of Medicine, Bachelor of Surgery, Diploma in Child Health, Member of the Royal College of Physicians of the UK, Fellow of the Royal College of - Royal Australasian College of Physicians, Doctor of Medicine, and Fellowship of the Heart Rhythm Society.

FURNESS SC: Thank you. You're an Honorary Professor in Paediatrics, Child and Youth Health at the University of Auckland?

WITNESS SKINNER: I am, yes.

FURNESS SC: Can you tell us what a paediatric electrophysiologist is?

WITNESS SKINNER: Yes. So, we deal with heart rhythm, just - so, I'm a children's heart specialist and deal primarily with the electrics. You might say that many of my colleagues deal with the plumbing, I'm the electrician. So, I deal mostly with heart rhythm disturbance, and for the last 15, 20 years I've had a major interest in sudden death syndromes and, as such, have developed and lead a national organisation which - whose core aim is to prevent sudden death in the young.

FURNESS SC: Thank you. Now, I think you've published a three-year, all core study of sudden death in 1 to 35-year-olds in Australia and New Zealand?

WITNESS SKINNER: Yes, one of the collaborative groups that did that, led by Chris Semsarian in Sydney and many other leading authors from Australia as well as New Zealand. This was the result of ten years work where we - ten years ago a multidisciplinary group - forensic pathologists, geneticists, laboratory scientists and so on - developed a best practice document for the investigation of young sudden death, which has since been ratified with the major colleges of pathology, cardiac society and genetics. And, based on that, we prospectively looked at all young sudden deaths between the age of 1 to 35 over all of Australia and New Zealand over a three-year period, and this was published in the New England Journal of Medicine in 2016.

FURNESS SC: I think you have particular expertise in the interpretation of ECGs in - or rhythm disorders in children, is that correct?

WITNESS SKINNER: Absolutely, I mean that's my core business, and having

a major interest in cardiac ion channelopathies, these are the conditions we'll hear more about later, I guess. Things like Long QT syndrome, I've become something of an expert in interpreting ECGs in these conditions.

FURNESS SC: Thank you. Professor, you provided a report at the request of the Inquiry dated 31 March 2019?

WITNESS SKINNER: Yes.

FURNESS SC: I tender that report, your Honour, together with the letter of instruction and CV.

EXHIBIT #Y REPORT OF PROFESSOR JONATHAN SKINNER DATED 31/03/19 TOGETHER WITH LETTER OF INSTRUCTION AND CV TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: Can I turn to you, Professor Kirk, your full name and work address?

WITNESS KIRK: Edwin Philip Enfield Kirk. My work addresses are Sydney Children's Hospital and the NSW Health pathology lab in Randwick.

FURNESS SC: And the current position you hold, Professor?

WITNESS KIRK: So, I'm - I've just recently stepped down as co-head of the Centre for Clinical Genetics and Sydney Children's Hospital, so I'm a senior staff specialist in clinical genetics there and a senior staff specialist in genetic pathology for NSW Health Pathology.

FURNESS SC: Do you hold any honorary positions?

WITNESS KIRK: I'm a Conjoint Professor at the University of New South Wales.

FURNESS SC: Thank you. Now, you initially trained in paediatrics, is that right?

WITNESS KIRK: Yeah, that’s right, the structure of training in genetics in Australia begins with paediatric training, always.

FURNESS SC: You completed your training in clinical genetics, when?

WITNESS KIRK: 1998.

FURNESS SC: Since that time, where have you largely worked?

WITNESS KIRK: In clinical genetics at Sydney Children's Hospital.

FURNESS SC: Now, you were you Head of Metabolic Diseases Service at the Sydney Children's Hospital from 1999 to 2011?

WITNESS KIRK: Yes, that's correct.

FURNESS SC: And you've continued clinical involvement in that area?

WITNESS KIRK: Yes.

FURNESS SC: And you're involved in a roster at the Children's Hospital Westmead in respect of that area?

WITNESS KIRK: Yeah, that's right. So, I'm currently on call for New South Wales and the ACT.

FURNESS SC: Hopefully not at the moment, Professor.

WITNESS KIRK: I've got someone covering me during the day today, yeah.

FURNESS SC: Thank you. Now, you undertook research in cardiac genetics at the Victor Chang Cardiac Research Institute and ultimately received a PhD in that area?

WITNESS KIRK: Yes, that's correct.

FURNESS SC: So, that's an area of particular research interest for you, is it?

WITNESS KIRK: Yes, that's right. That was primarily in congenital heart disease and most of my research has been in congenital heart disease, but my cardiac clinical practice has mainly been in cardiomyopathies and encephalopathies.

FURNESS SC: With children?

WITNESS KIRK: Children and adults.

FURNESS SC: Now, I think your other areas of research have included metabolic diseases?

WITNESS KIRK: Yes, that’s correct.

FURNESS SC: And epileptic encephalopathy?

WITNESS KIRK: Yes.

FURNESS SC: Now, you're also a genetic pathologist?

WITNESS KIRK: Yes, that's correct.

FURNESS SC: What's the difference between a genetic pathologist and a clinical geneticist?

WITNESS KIRK: A clinical geneticist is a clinician, so someone who sees people clinically. So, it might involve examining a child or an adult, interpreting family history and ordering and interpreting genetic tests. A genetic pathologist is involved in the laboratory diagnosis of genetic diseases and there are many facets to that role, but an important part of that is interpreting and reporting on genetic data. So, they are closely related but separate specialties, and I'm dual-trained in both.

FURNESS SC: I think you're currently the Chief Examiner in Genetic Pathology for the College?

WITNESS KIRK: For the Royal College of Pathologists of Australasia, yes, that is correct.

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

WITNESS BUCKLEY: My name - my full name is Michael Francis Buckley. I - my professional address is the Randwick Genetics Laboratory at the Prince of Wales Hospital site in Randwick, Sydney.

FURNESS SC: What position do you currently hold, Doctor?

WITNESS BUCKLEY: I'm the Clinical Director of that laboratory.

FURNESS SC: The?

WITNESS BUCKLEY: The Clinical Director of the laboratory. So, I'm a genetic pathologist in charge of the Randwick Genetics Laboratory.

FURNESS SC: Thank you, and your qualifications, Doctor?

WITNESS BUCKLEY: I have a Bachelor of Human Biology, a Bachelor of Medicine and Surgery, a PhD, I'm a Fellow of the Human Genetics Society of Australasia, I'm a Fellow of the Royal College of Pathologists of Australasia, I'm a Fellow of the Royal College of Pathologists in the United Kingdom, and I'm a Fellow of the Faculty of Science of the Royal College of Pathologists of Australasia.

FURNESS SC: And your PhD was in the field of molecular genetics, is that right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: Thank you. Now, you say you're certified as an associate cytogeneticist by the Human Genetics Society of Australasia. What is a cytogeneticist?

WITNESS BUCKLEY: A cytogeneticist is one of the three sub-branches of laboratory genetics that studies the structure of chromosomes and looks at

numerical and structural variance of chromosomes as a way of making genetic diagnoses.

FURNESS SC: Thank you. Can I turn to you, Dr Colley, your full name and professional address?

WITNESS COLLEY: Alison Fiona Colley, Liverpool Hospital, South Western Sydney Local Health District.

FURNESS SC: Your current position, Doctor?

WITNESS COLLEY: Director of Clinical Genetic Services.

FURNESS SC: For that local health district and various others?

WITNESS COLLEY: Yes, Southern NSW Local Health District and Murrumbidgee Local Health District and previously, also, Central Sydney Local Health District, though I'm - they now have a separate head.

FURNESS SC: Thank you. And your qualifications, Doctor?

WITNESS COLLEY: Bachelor of Medicine, Bachelor of Surgery, Fellow of the Royal Australasian College of Physicians in paediatrics, Human Genetics Society of Australasia Certified Clinical Geneticist and Master of Medical Science in Epidemiology and Biostatistics.

FURNESS SC: Thank you. Now, 1991, you were a staff specialist clinical geneticist at the Hunter Area Health Service?

WITNESS COLLEY: Yes, I had just come back from training in the United Kingdom and I took on a consultant position as a staff specialist in the fairly new, developing genetic services at Waratah Hospital to service the Hunter area - Hunter area.

FURNESS SC: You were there for about five years?

WITNESS COLLEY: Yes.

FURNESS SC: And we'll come back to your experience there, Doctor. Now, as I indicated earlier, you are also a dysmorphologist?

WITNESS COLLEY: Right.

FURNESS SC: What does that do?

WITNESS COLLEY: Basically, it's a field of, or an area of clinical genetics where we're particularly trained and interested in understanding what a person looks like and their particular features, and how that might relate to their genotype. So, it's a clinical science part of clinical genetics and related to what might be considered outside the normal for that family or for that ethnic

background and those particular families where the person comes from. So, trying to look for evidence on the outside of a person that might indicate what's happening with their genotype.

FURNESS SC: Thank you. Can I come back to you, Professor Buckley? Each of you contributed to a joint report at the request of the Inquiry, that's right? And I think there are a number of corrections that we need to make. So, perhaps if I can start with you, Dr Buckley? Appendix 1 of the report, this is on page 10 of the report, have you got that in front of you?

WITNESS BUCKLEY: Yes, I do.

FURNESS SC: Perhaps that can come up on the screen.

WITNESS BUCKLEY: I have that in front of me.

FURNESS SC: We'll wait for everyone to see it on the screen. At page 10 and then, the second last paragraph, the last sentence "Of note, no member of this family has had a Brugada pattern observed on ECG". Is that how you pronounce it?

WITNESS BUCKLEY: Yes, that's how you pronounce it.

FURNESS SC: In fact, I think it's the case that only Patrick and Laura had an ECG, is that right?

WITNESS BUCKLEY: Yes, where an ECG had, had been performed, no member of the family had had a Brugada pattern.

FURNESS SC: So that's in reference to Patrick and Laura?

WITNESS BUCKLEY: Yes.

FURNESS SC: I think does it also refer to Ms Folbigg? She had an ECG as well, didn't she?

WITNESS BUCKLEY: Yes, it does - yes.

FURNESS SC: Thank you. Appendix 8, which is page 24, the first sentence, "The MYH6 variant is present in Kathleen, Caleb, Patrick and Laura", in fact it was not present in Patrick, is that right?

WITNESS BUCKLEY: That's correct, it's an error and I apologise for that.

FURNESS SC: That's all right, thank you. If we come back to appendix 3 on page 16, under the heading "Summary", you set out that the variant is classified or categorised as likely benign and you refer to BP1, BP6 and BS1. I think you wish also to include BS2?

WITNESS BUCKLEY: Yes, the strong benign criterion BS2 should be included

there as Ms Folbigg has no evidence of being affected by that disorder.

FURNESS SC: Your Honour, we'll come to the detail of what all this means, but BS2 is effectively observed in a healthy adult individual. And the fact that it was observed in Ms Folbigg is your reasoning for including BS2?

WITNESS BUCKLEY: That's correct.

FURNESS SC: And as a result of including BS2, the categorisation of "likely benign" should be changed to "benign"?

WITNESS BUCKLEY: Yes, that's correct.

FURNESS SC: Appendix 5 at page 19. The summary refers to PM2 and BP4. Again you wish to add BS2?

WITNESS BUCKLEY: Yes, for the same logic that this in particular is a paediatric onset severe intellectual disability disorder and I'm reliably told that Ms Folbigg does not have that disorder.

FURNESS SC: Accordingly, that's a matter to be taken into account to determining the categorisation of the variant, that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: So you would categorise it as likely benign, rather than variant of uncertain significance?

WITNESS BUCKLEY: That is correct.

FURNESS SC: Thank you. I take it, Professor Kirk and Dr Colley you agree with the amendments that have just been made to your joint report?

WITNESS COLLEY: I do.

WITNESS KIRK: Yes.

FURNESS SC: I'm sorry, you--

WITNESS COLLEY: I do.

FURNESS SC: Thank you. Professor Kirk? You need to answer--

WITNESS KIRK: Yes.

FURNESS SC: Thank you.

EXHIBIT #Z JOINT REPORT OF PROFESSOR KIRK AND DRS BUCKLEY AND COLLEY TOGETHER WITH LETTERS OF INSTRUCTION AND CVS TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: In addition, Dr Colley, you provided a short report dated 26 November 2018 summarising your previous involvement with respect to the Folbiggs and providing advice on medical advances?

WITNESS COLLEY: Yes.

FURNESS SC: I tender that, your Honour.

EXHIBIT #AA REPORT OF DR COLLEY DATED 26/11/18 TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: And Dr Buckley you provided a report on progress in relation to genetic advances, if I can reduce it to that simple concept, dated 25 February 2019?

WITNESS BUCKLEY: That is correct.

EXHIBIT #AB REPORT OF DR BUCKLEY DATED 25/02/19 TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: I also tender the Genetics Tender Bundle, your Honour.

EXHIBIT #AC GENETICS TENDER BUNDLE TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: Can I turn first to you, Dr Colley, and if we can start with page 4 of the report, which I'll refer to as the Sydney report. Do you have that in front of you?

WITNESS COLLEY: I do.

FURNESS SC: At section 3 of page 4, you refer to in the heading "The Phenotype in the Sibship". Perhaps you can explain to us what a phenotype is, to begin with?

WITNESS COLLEY: A phenotype is the observable characteristics of a person and I think all of us would immediately think of observable characteristics being facial features. We also must remember it would include things like the size of a person and this is particularly important with children where height, weight and head, head measurement relevant for each particular age is very important. Physical malformations, which can be present and sometimes called birth defects forms part of a phenotype. They might be things like extra fingers and toes or something more subtle about the facial features regarding the ears or the nose, the creases on the palms of the hands or the nails on the, on the fingers and the toes. These would be called morphological features, when something is different than expected, that's when we call it dysmorphology.

But as well as the morphology or physical features, a phenotype includes other

observable characteristics, such as a behaviour. You can have a behavioural phenotype. Some children with particular disorders are very irritable when they're young. Some are quite aggressive. Some have self-injurious behaviour. Some don't sleep. All these are observable characteristics. Other people would also include things like personality. Certainly, cognitive understanding, whether someone is intellectually disabled or not is an observable phenotype, so observable characteristic, so it makes up part of the phenotype.

FURNESS SC: Thank you. You say in the second paragraph that a characteristic of genomic testing in complex disease is that the laboratory data must be interpreted in the context of the clinical presentation. The clinical presentation goes beyond what is observable?

WITNESS COLLEY: The clinical presentation might include, might include someone's illness or for example, a syncopal episode, where someone faints--

FURNESS SC: Perhaps you need to explain syncopal, is S-Y-N-C-O-P-A-L?

WITNESS COLLEY: Yep.

FURNESS SC: What is a syncopal episode?

WITNESS COLLEY: A syncopal episode is when someone loses consciousness and would drop to the floor. The most common cause is what we call a vasovagal episode.

FURNESS SC: Which is?

WITNESS COLLEY: When the vagus nerve is overstimulated, usually an event of a fright or a sudden highly emotional event and the heart rate slows and the blood pressure goes low and a person loses consciousness and drops to the floor. So that would be an observable characteristic, but we wouldn't really call that a phenotype, because it's something that just happens intermittently.

FURNESS SC: All right, thank you. Over the page you refer to various documents, 26 in total, although 1 and 2 are more comments rather than documents and then the conclusions that you have drawn from each of those documents set out in 1 to 15, do you see that in the report?

WITNESS COLLEY: I can.

FURNESS SC: Can I just draw your attention firstly to the notes in the Hunter Genetics file, which is tab 74? If we can have 74 on the screen? Thank you. Do you have that?

WITNESS COLLEY: Yes.

FURNESS SC: These are records that you made, is that right?

WITNESS COLLEY: That's correct.

FURNESS SC: And tell us the context in which you made them?

WITNESS COLLEY: In 1991, following the death of Patrick, Mrs Folbigg's second child, her and her husband went to see their general practitioner because they decided they wanted to have a third child and they were naturally very anxious as to what might happen to that child and so their general practitioner referred them to a number of people, but including the genetics service and they saw myself. So they came along and you can see there on 12 November 91 they were seen by myself. At that time I basically had a discussion with both of them, Mr and Mrs Folbigg. I collected information, drew a family tree, realised that I needed - in order to answer their question about--

FURNESS SC: Let me just stop you and slow you down for a moment.

WITNESS COLLEY: Yep.

FURNESS SC: You said you collected information for a family tree?

WITNESS COLLEY: Yes, to do that I just spoke with them and asked them about their family members.

FURNESS SC: So each of Craig and Kathleen Folbigg?

WITNESS COLLEY: Yes.

FURNESS SC: And you asked them about their family members?

WITNESS COLLEY: Yes.

FURNESS SC: And you recorded that in the tree, did you?

WITNESS COLLEY: Yes.

FURNESS SC: And the tree is on page 2, if we can have page 2 on the screen of the document?

WITNESS COLLEY: Yes.

FURNESS SC: That's a tree?

WITNESS COLLEY: That is. We call it a family tree. I guess the - another name is a pedigree.

FURNESS SC: What did you learn from each of Ms and Mr Folbigg that you recorded on the tree?

WITNESS COLLEY: Well, first of all, I should note that in this tree it includes Sarah and she wasn't born when I drew this tree. So the file is kept and the next time this couple came to the Hunter Genetics the person they saw would have added in Sarah. So I did the rest of the tree there. So what I've got is that Kathleen was adopted and you can see there's no family - there's no siblings, parents, aunts and uncles that have been put on her side of the family tree. You can see Craig is not adopted. You can see that he has brothers and sisters and mother and father there. His mother was already deceased at age 43, from what was thought to be a cerebral haemorrhage. His father had had bypass surgery.

I've got noted there too that he spoke to me about - because I asked specific questions in gathering this information, obviously I'd like people to tell me as much as they can, because sometimes I have to direct them to ask specific questions otherwise you'd miss out on data. So I asked him about had anyone died young in his family and he told him that his brother had had a child who died young, but he didn't know the cause and he, he wasn't sure about what age they died at and - or whether they had any illnesses. So there was very little detail, there's really no detail, but there had been another baby who had died at some time.

FURNESS SC: A nephew, he thought?

WITNESS COLLEY: A - he thought it was a nephew.

FURNESS SC: What does the + NND or T NND mean?

WITNESS COLLEY: It's actually a cross for death and NND is a neonatal death.

FURNESS SC: I see and that's what you've just been referring to?

WITNESS COLLEY: Yes. Sometimes the term neonatal death is quite strict. You might use the first 28 days of life. Here I know from speaking to him he really wasn't sure of when, but, but young.

FURNESS SC: You also had the opportunity to meet with Mr Folbigg's sister?

WITNESS COLLEY: Carol and her husband Robert.

FURNESS SC: And that's referred to in the tree?

WITNESS COLLEY: Yes.

FURNESS SC: Was there anything observable about Mr Folbigg or his sister that caused you to draw any conclusions?

WITNESS COLLEY: Only that they were both of normal stature, normal facial appearance, they were not what I'd call dysmorphic. They were normal white Australian, Caucasian looking people. They also appeared to have normal

intelligence and their behaviour was appropriate and normal for a consultation.

FURNESS SC: Did you draw any conclusions from the information that you received from Mr Folbigg in relation to contributing to knowledge about the death of the children?

WITNESS COLLEY: He was very anxious, but not giving me any information about why they had died other than his understanding from the autopsy reports that it was classed as a sudden infant death syndrome.

FURNESS SC: But my question was more directed to your expertise as a clinical geneticist, Dr Colley. You've indicated what you said about their appearance, which goes to any question of dysmorphology. Was there any other matter that came to your attention as part of that process that enabled you to draw any conclusion in your areas of expertise?

WITNESS COLLEY: Only that Mr Folbigg and his sister and the information they gave me was that there was no known genetic disorder in the family, there was no known inherited disorder in the family. There was no known disorder that caused children to be unwell and spend time in hospital and, and not grow properly or anything like that.

FURNESS SC: Thank you. Coming back to your report on page 5, at number 23 you refer to photographs of the four Folbigg children?

WITNESS COLLEY: Yep.

FURNESS SC: And can I show you tab 73? Are they the photographs you were referring to?

WITNESS COLLEY: Yes.

FURNESS SC: Do you now recall the circumstances in which those photographs came to your attention?

WITNESS COLLEY: There were some photographs that came to my attention when I saw Mr and Mrs Folbigg, because part of what we do as a clinical geneticist is that we request photographs. As you know, I didn't see any of the children and the first time I met Mr and Mrs Folbigg, Caleb and Patrick had died, so I, I couldn't see them. So one of the things when trying to assess morphology is to have a look at photographs, as well as to read widely on the reports from other people who did see them personally. But the other two children Sarah and Laura I only saw the photographs that were in the media.

FURNESS SC: Thank you. Coming back to page 5 of your report, you refer to the conclusions that you drew from the documents referred to above and your meetings with Mr and Mrs Folbigg, that's right?

WITNESS COLLEY: Yes.

FURNESS SC: Your Honour, just for the benefit of those at the bar table, those assisting your Honour have provided or prepared a document setting out the respective tab number to each of these documents. I'm not sure if it's been provided, but if it hasn't it will be now. It might be of assistance. So each of them is in evidence. Firstly, you say there was no evidence of pregnancy related complications, all children had good Apgar scores at birth and normal birth weights?

WITNESS COLLEY: Yes, I think that's important because we do know children who are, have a genetic condition. Quite often there can be some concerns during the pregnancy, extra fluid around the babies like polyhydramnios, poor movements, those sorts of things, poor growth in pregnancy, and there was no evidence of that on anything that I read. The Apgar score is a score given to all children at one minute and five minutes after birth. It's made up of five components, but it's a good overall indication of the robustness or healthiness of that baby at birth, and they all had good Apgar scores, and they all had height, weight and head circumference within - that we have, that were well within the normal centile lines for the time at birth.

FURNESS SC: You say there was no congenital malformations noted at birth, and that's noted by the various medical and nursing people who were there--

WITNESS COLLEY: Yes.

FURNESS SC: -- at the relevant time?

WITNESS COLLEY: Yes, and also of course on the autopsy because malformations will still be there when the babies were deceased, and it would have been noted by the anatomical pathologist.

FURNESS SC: Each child had newborn screening reported as normal?

WITNESS COLLEY: Yes.

FURNESS SC: You're referring there to Professor Wilcken's work?

WITNESS COLLEY: Yes.

FURNESS SC: The fourth matter is the dysmorphic features and you've given evidence as to what that means?

WITNESS COLLEY: Yes.

FURNESS SC: Again, that's by reference to the many, particularly in relation to Patrick, the many medical officers and nursing people and the like who had the opportunity to observe and record their observations?

WITNESS COLLEY: Yes. I think it's worth saying, and some people might say well if you're not a dysmorphologist or you're not a clinical geneticist could you really recognise dysmorphic features? Well I think the answer to that is

yes. You mightn't know what to call them and certainly many nurses, midwives, GPs who saw these children might not have used the sort of terminology I would use, but they would say things like, "Mm, this child looks a little unusual" or "doesn't look like parents" or "looks different than other people". So yes, there would have been some notes if there was any dysmorphic features.

FURNESS SC: And you saw none?

WITNESS COLLEY: Sorry?

FURNESS SC: You saw no notes to that effect?

WITNESS COLLEY: No, no notes, and again the anatomical pathologist who's very experienced at looking at children did not note any dysmorphic features.

FURNESS SC: Thank you. You then refer to development being reported as normal which I think you've covered in your evidence to date, and all children were thriving at the time of their unexpected event.

WITNESS COLLEY: Yes.

FURNESS SC: Stopping there, you're referring here to prior to Patrick's ALTE aren't you?

WITNESS COLLEY: Yes.

FURNESS SC: Not post-ALTE?

WITNESS COLLEY: No, referring to Patrick up to four and a half months when he had that event.

FURNESS SC: You've mentioned the normal growth parameters and none of the children had a surgical operation or procedure. Number 10 you say none of the children were admitted to hospital with a significant medical problem, and clearly that's before Patrick's ALTE?

WITNESS COLLEY: Yes, yes.

FURNESS SC: Similarly, none of the children were on continuous medication before Patrick's ALTE?

WITNESS COLLEY: Yes.

FURNESS SC: Number 12 you say none of the children were documented to have more than eight respiratory infections a year.

WITNESS COLLEY: Mm-hmm.

FURNESS SC: That was based on the medical records and the other

documents you've referred to?

WITNESS COLLEY: Yes.

FURNESS SC: Why is the number eight relevant?

WITNESS COLLEY: Well young children, particularly in the first year or so of life, have a lot of upper respiratory tract infections and, you know, some parents think oh my goodness my child's always got a cough or a cold, is that normal? And so studies have been done and I think I presented a couple of papers, looking at just what is normal for the number of respiratory tract infections per year for a child at different ages. I think eight is a very reasonable number. Some people would say six, some people might say up to ten, but eight is what's accepted.

FURNESS SC: Your Honour there were a number of articles that had been provided by the witness to support her evidence in relation to eight and I'm happy to make them available to my friend, and ultimately as with the previous hearing, those assisting your Honour will put together a bundle of documents referred to and ultimately tender them, so we'll make those available.

WITNESS COLLEY: Can I make one other comment there?

FURNESS SC: Certainly.

WITNESS COLLEY: That is about respiratory tract infections, and I think what's really important is the fact that these children who were not admitted to hospital with more serious infections. We don't have any evidence of meningitis, encephalitis, peritonitis, widespread skin infections, whether it be pastoral infections from a bacterial infection, or candidiasis from fungal infections, because children who are born or who have genetic predisposition to infections, one would have expected to see more serious infections than just your usual running nose and cold.

FURNESS SC: And you didn't see any of that in the material?

WITNESS COLLEY: No. I think one child, Laura, the last child, as we know, lived longer to 20 months, so she had a longer period of time in which to get infections, and so it's quite usual in that case you would see some other infections, but we only saw, that I read about, was one episode of gastroenteritis, one episode of croup, but nothing that I would consider that would raise alarm bells for me with a child having a significant predisposition to infections.

FURNESS SC: Number 13, you refer to the testing that again was done by Professor Wilcken?

WITNESS COLLEY: Yes.

FURNESS SC: The results of that are available in the material, your Honour.

That's already before the Inquiry. Number 15, you say all children had autopsy examinations with no medical cause of death determined. Stopping there, you understand that with Patrick of course his condition was compromised by his ALTE--

WITNESS COLLEY: Yeah.

FURNESS SC: --and the consequences, you understand that?

WITNESS COLLEY: Yes, yes.

FURNESS SC: In relation to Laura, myocarditis was found on autopsy?

WITNESS COLLEY: Yes. With Laura, my reading of that autopsy report, or the original one was that I thought the anatomical pathologist did not think that the myocarditis was sufficient to cause the actual death, but I do know that there are other people who have, other pathologists, who have looked into that and given their opinions as well.

FURNESS SC: You'd clearly defer to the pathologist as to what they saw and interpreted?

WITNESS COLLEY: Absolutely.

FURNESS SC: In addition, the Inquiry has heard evidence that the children all died during a sleep period and that they were supine when found. Do those two factors affect your opinion in respect of the phenotype?

WITNESS COLLEY: No.

FURNESS SC: Do they add to it?

WITNESS COLLEY: No. I - it's a statement and it's true and from what I understand it's been reported that that's how they were found. I don't think that makes a genetic disorder more or less likely.

FURNESS SC: Can I turn to you Professor Skinner if you're still with us. Professor, can I ask you to have a look at your report and perhaps if page 4 of Professor Skinner's report can be put on the screen. Professor, you have had the opportunity to look at whatever clinical information is available of a cardiac related nature in respect of the children? That's right?

WITNESS SKINNER: Yes, I have, yes.

FURNESS SC: You've recorded your opinions in the report beginning on page 4?

WITNESS SKINNER: Yes.

FURNESS SC: In relation to Craig, as you state there's been no cardiac

testing, that's right?

WITNESS SKINNER: That's right, as far as I know.

FURNESS SC: Similarly with Caleb?

WITNESS SKINNER: Yes.

FURNESS SC: Turning to Patrick, I want to take you to some documents that we have concerning Patrick and the first is at tab 3. That's an ECG that was done on 18 October, is that right?

WITNESS SKINNER: That's right.

FURNESS SC: You have interpreted that as telling you what about it?

WITNESS SKINNER: So it looks like a perfectly normal ECG for age, the heart rate is normal, the heart rhythm is normal. The various features of it were all entirely normal. In particular, there are no features to suggest Long QT syndrome, which is one of the commoner causes of sudden death in childhood. There's nothing to suggest Brugada syndrome, and there's no - which is another condition which more rarely causes sudden death in the young, and there's no - in particular there's no conduction defect between the top and bottom of the heart. You can see everything's joined up nicely. There's nothing to suggest that any of the chambers are enlarged.

FURNESS SC: Just turning back to tab 2, that was another ECG I think that was carried out with respect to Patrick?

WITNESS SKINNER: Yes, this is a little more difficult to interpret because the reproduction's not very good. It's actually nine leads I seem to recall rather than 12 leads, and there's quite a lot of noise on it--

FURNESS SC: Page 3 of that document.

WITNESS SKINNER: --back, you know, artefact, you would say electrical noise from machinery and the like. But what you can conclude is that the QT interval is normal so there's no evidence of Long QT syndrome. The rhythm and conduction system seems to be functioning normally, and there's no features in either of these ECGs that would suggest an ongoing or acute inflammation where you get changes called ST segment changes and things like that. I think it all looks normal.

FURNESS SC: Then if we can go to tab 5. Perhaps if that's on the screen, tab 5.

WITNESS SKINNER: That's, that's part of the same ECG. It's all - I think it's just been cut up into strips and stuck on the clinical notes.

FURNESS SC: And tab 5--

WITNESS SKINNER: The echocardiogram, yeah.

FURNESS SC: That's what you're referring to, it's on the screen?

WITNESS SKINNER: Okay, I've got you there. So that I believe is the echocardiogram report, yes, so obviously this is a report of the echocardiogram, this is an ultrasound examination of the heart and--

FURNESS SC: Sorry, let me just stop you there.

WITNESS SKINNER: Yes?

FURNESS SC: Can you explain again what an echocardiogram is?

WITNESS SKINNER: Well it's, it's a scan. We are all familiar with ultrasound scans that are done during pregnancy to see the baby. This is similar where jelly is applied to the chest and ultrasound is used to image the heart, and there are standard things that are measured, so the heart is described as structurally normal and so it's just a normal study at the bottom. But in particular, and of importance with respect to cardiomyopathy, a disorder of the heart muscle, the left ventricle size is described as normal, left atrial side is described as normal, normal size and function. So essentially a normal echocardiogram.

FURNESS SC: I think you say in your report that the echocardiogram showed no evidence of heart muscle disease?

WITNESS SKINNER: That's right.

FURNESS SC: In relation to Sarah, you haven't seen any cardiac test results, that's right?

WITNESS SKINNER: That's right.

FURNESS SC: Can we then turn to Laura. Perhaps if we can have tab 23 on the screen. This is a report from Dr Seton?

WITNESS SKINNER: That's right.

FURNESS SC: It refers to an overnight ECG in August 1997 and again in February 1998. Do you see that in the report?

WITNESS SKINNER: Yes.

FURNESS SC: What conclusions do you view, or do you draw from this report?

WITNESS SKINNER: Well, Dr Seton reports that the - there's a single lead ECG. So, this is only just one of the normal 12 leads and it's just really useful

to monitor rhythm, there isn't much else that one can do with an ECG like that. You can't, for example, exclude or diagnose Long QT syndrome or Brugada syndrome, but you can tell if the rhythm is stable, if it's going too fast or too slow, for example, or it becomes very irregular. And Dr Seton makes the comment that the ECG was normal throughout the polysomnography, the, the study of the sleep overnight on those two occasions. And there were some of those extracts of rhythm strips that were provided, and I reviewed those rhythm strips and was quite happy that the rhythm is normal sinus rhythm - that's just the description of normal rhythm, with no pauses, no abnormal extra beats and no evidence of any conduction system disorder.

FURNESS SC: Thank you. Now, just before we leave Laura and coming to page 5 of your report, if we can have that on the screen, you were asked specifically about the significance of the agonal rhythm--

WITNESS SKINNER: Yes.

FURNESS SC: --which was identified by one of the ambulance officers in respect of Laura? Do you recall that?

WITNESS SKINNER: Yes. Yes, I do. I, I don't know if, if the actual rhythm strip is - can be shown there, can it? Because it would be easiest to, to talk to it. But, essentially, what I was able to review was the, the rhythm strip - similarly a rhythm strip recorded by the ambulance officers during the resuscitation attempt and--

FURNESS SC: Perhaps if I can just stop you for a moment, Professor, and we can have tab 20 - page 47 at the bottom of tab 20 might be what you're referring to? This is the ambulance officer's statement.

WITNESS SKINNER: Yes. Yes, okay, so, it's - reproduces poorly, but that does fine. So, these individual lines you can see are consecutive, so they're run one after the other. So, you can see at the top there, if - that - that's good, if you just leave that like that. Now, it's time along the bottom and it's amplitude going up and down. So, each of those squiggly lines represents a deflection. Now, 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 at about 100 or 110 beats per minute.

But you can see in between some of those chest compressions, if we go then down to line - one, two, three, four - five, you see there's a pause at the beginning and it says "12:10:35". There is a - an apparent beat. It's a broad signal which doesn't look nice and narrow like the other beats did on Patrick's ECG earlier on, and that would be a typical beat from an agonal rhythm. What it means is there's an electrical activity in the heart, it's clearly very abnormal and, and it's typically not associated with a pulse, the heart doesn't actually contract. So, that agonal rhythm is what we're discussing, I think.

FURNESS SC: And you drew a conclusion about the presence of that rhythm, which you set out at the--

WITNESS SKINNER: So - yes. So, the usual mode of death for a cardiac encephalopathy, like Long QT syndrome or these heart rhythm disorders, is a very rapid rhythm called ventricular fibrillation. Now, usually, if there is a cardiac arrest - and this applies to coronary artery disease, coronary artery disease is a cardiac arrest in the street - what you see is a rapid heartbeat, so rapid that the heart is like having a seizure, it's not contracting. Now, normally when you arrive at such a cardiac arrest that rhythm would still be running and you hope that you're able to apply electricity, give a shock and return them to a normal rhythm.

Normally, when we see a rhythm like this, it's most typically associated with the primary rhythm problem being asystole, in other words, the heart stopping. We see this in neurological disorders and children who have died from overwhelming sepsis and from other multisystem problems and it's the last thing that happens to them. Basically, the heart gives up. But what - I, I wasn't able to be completely confident about that. I think that this is usual for what you see in a non-cardiac death, but I think sometimes ventricular tachycardia or ventricular fibrillation can stop spontaneously and then, the heart being so sick, it just does these final death throes or agonal rhythm. So, I'm - I think my conclusion was that this rhythm makes a non-cardiac death more likely than one from a primary cardiac arrhythmia, but I don't think that's a conclusive thing.

FURNESS SC: Thank you. Professor Kirk, did you want to say anything in respect of that?

WITNESS KIRK: No, no.

FURNESS SC: Now, can we turn over to page 6 of your report? This is in relation to Kathleen.

WITNESS SKINNER: Yes.

FURNESS SC: You've said that you haven't interviewed Kathleen, however you have read whatever documents have been provided to you in respect of her cardiac related health?

WITNESS SKINNER: Yes.

FURNESS SC: Now, if we can have tab 35 on the screen? Now, this is an EEG. What's an EEG?

WITNESS SKINNER: Well, that's electroencephalogram, so that's the brainwaves. That's definitely not my area of expertise.

FURNESS SC: In relation to the report, this EEG report, the history is of "recent pregnancy with an episode of syncope" - how do you pronounce that?

WITNESS COLLEY: Syncope.

WITNESS SKINNER: Syncope.

FURNESS SC: Syncope, thank you. We've been told by Dr Colley that that's, effectively, fainting?

WITNESS COLLEY: Yes.

WITNESS SKINNER: That's right. I, I wanted actually - thank you for the opportunity, I wanted to just take something Dr Colley had said. She mentioned that vasovagal syncope - and she gave an example of a fright, where you drop to the floor. I just want to correct that, if I may, because vasovagal syncope doesn't usually follow a fright. That, that is something you might see on a black and white sitcom comedy on the - but it's not actually what happens. If you have a fright, there's typically an adrenergic surge and I would be more worried about heart rhythm disturbance.

But, yes, so syncope, the commonest cause of syncope is vasovagal fainting, and this is the classic situational thing where you get - where you're too hot or you're too dehydrated. It commonly occurs during pregnancy, for example, it might happen when you have a blood test, that sort of thing.

FURNESS SC: So, do you draw any conclusion from this report that tells you anything about Ms Folbigg's heart?

WITNESS SKINNER: Not so much the report itself. I would just say that, that it was an EEG following a blackout during pregnancy and that I think fainting is common in pregnancy. It's usually a haemodynamic or a blood pressure issue and it's rarely due to a heart rhythm disturbance, although of course that is possible. They did say that she had some minor seizure activity and, and this can occur with a common faint as well. Basically, if your brain is short of oxygen, you can have a seizure. So, I think the - if you blackout first due to a, a failure of getting enough blood into your brain, you can go on and have a little seizure. So, I wouldn't take it as meaning she has epilepsy.

FURNESS SC: Thank you. Can we turn to tab 39? We're moving along here to 2004. Do you see that it what appears to be--

WITNESS SKINNER: Yes.

FURNESS SC: --a form completed by Ms Folbigg?

WITNESS SKINNER: Yes. Yes, and I, I noted, in particular, it's a self-filled health report, signed by Kathleen, where she reports no seizures at one--

FURNESS SC: Now, just let me stop you there. You see there's a box down the bottom of the page. On the side it says, "Health History" and then there's a heading "You", and then there's a heading "Your Immediate Family". Do you see those on the document?

WITNESS SKINNER: Yes.

FURNESS SC: And you're referring to the heading "You", that's right?

WITNESS SKINNER: Yeah. That's right, and--

FURNESS SC: And that by--

WITNESS SKINNER: The fourth one down.

FURNESS SC: --striking out the circle, you're interpreting that as being she is saying she doesn't have any of those things, is that right?

WITNESS SKINNER: That's correct, yes.

FURNESS SC: And one of them is "Epilepsy/seizures"?

WITNESS SKINNER: Yes.

FURNESS SC: And another is "Heart disease"?

WITNESS SKINNER: Yes.

FURNESS SC: Then, if you turn to the next column, which is "Your Immediate Family", similarly, there is a strikeout in respect of "Heart disease" and concerning "Epilepsy/seizures", there is, I think, a tick--

WITNESS SKINNER: Yes.

FURNESS SC: --and reference to "Second child"?

WITNESS SKINNER: Indeed, yes.

FURNESS SC: And that is consistent with the reference to Patrick, isn't it?

WITNESS SKINNER: Yes.

FURNESS SC: Thank you. So, you interpret that as being that she, at this stage, had no seizures?

WITNESS SKINNER: Correct.

FURNESS SC: Does that support the view you formed in respect of the earlier document, which was admittedly some years earlier, that the fainting episode was not related to a seizure-related condition?

WITNESS SKINNER: Yes, I, I took it that she, herself, interpreted the previous fainting episode as being a faint and, and not a seizure.

FURNESS SC: Thank you. Can we have tab 42? This is in relation to your

next entry, in 2009. Now, do you see at the top of the page, 8 March 2009, there's a nursing entry?

WITNESS SKINNER: Yes.

FURNESS SC: Can you read that for us?

WITNESS SKINNER: So, I - my interpretation was that she presented to the clinic complaining of dizziness, which she said happens every day, two to three times a day. And she mentions that - something about hitting her head when she fell, some four weeks ago. So, I, I, I felt that it was unlikely these events were cardiac related. That was my impression, at, at any rate.

FURNESS SC: What was it about those events that enabled you to form the opinion that they weren't cardiac related?

WITNESS SKINNER: Well, for, for a start, she'd just hit her head and she was complaining of regular dizziness. So, that sort of thing is much more likely to be related to some sort of head injury, feeling generally unwell. In general, intermittent dizziness isn't a likely sign of heart rhythm problems. You're much more likely to present with a sudden sensation of your heart going very, very fast or you simply hit the ground unconscious because there's just no blood getting to your head.

FURNESS SC: Hence your conclusion that it's unlikely they were cardiac related?

WITNESS SKINNER: Indeed.

FURNESS SC: Now, if we can have tab 40 on the screen? This is your next entry in May 2008?

WITNESS SKINNER: Yes.

FURNESS SC: Do you see there, there's a "Presenting problem" or "Provisional diagnosis"?

WITNESS SKINNER: Yes. So, here there was an alleged collapse in her cell after vomiting, and the notes describe "Collapse was after a vomit and on" - that's "OE", that means "on examination", "the patient was lying supine and she was diaphoretic", meaning she was sort of sweaty. She had a low blood pressure and her pulse was "attenuated", she had a weak pulse. So, this would be typical of a, a person who's got a low blood pressure, a low circulating blood volume, typically from dehydration or gastroenteritis or something like this. And I see at the bottom there that they recommended "Gastrolyte oral rehydration solution", which would be consistent with that impression. So, if she did have a collapse, I think it's very likely that it was related to the fact that her blood pressure was low from dehydration.

FURNESS SC: Thank you. Now, if we can go to 46? Now, that's an

echocardiography report?

WITNESS SKINNER: Yes.

FURNESS SC: Dated June 2011. Now, is that--

WITNESS SKINNER: Yes.

FURNESS SC: --likely to have been the report that followed on from the ECG she had in respect of that episode, is that right, or separate?

WITNESS SKINNER: I think it might be a separate episode from 23 June. Again, she had another funny turn, which I can talk about in a moment, but the echocardiogram report says that she was complaining of chest pain and dyspnoea on emotional stress. So that's, that's what they say is the reason for the study, but again the left ventricular systolic function, that's the squeeze factor or, or how well the heart is contracting is described as normal. The normal left ventricular volume, normal right ventricular volume and normal right ventricular function. Indeed everything is described as normal. So this we can take as excluding the evidence of cardiomyopathy or heart muscle disease.

FURNESS SC: Thank you. Can we go back to tab 44? This is progress notes in January 2011 and there's reference halfway down to episode of chest pain?

WITNESS SKINNER: Right.

FURNESS SC: Can you see that?

WITNESS SKINNER: I can't read it at the moment.

FURNESS SC: It's a dot point on the--

WITNESS SKINNER: Yes, "Episode of chest pain that occurred, occurred at rest while sitting, moderately severe". So I didn't refer to this in my report specifically I think, but she presents - she presented earlier in 2011 to the Registrar at - cardiology Registrar at Westmead Hospital, following this kind of atypical chest pain. Atypical meaning that not typical for angina. So she's describing pain there. It's not the sort of thing that you normally get with a cardiomyopathy or an inherited heart condition, but it, it, it is what it is.

FURNESS SC: If we can turn then to tab 45, this is the Registrar you were speaking of?

WITNESS SKINNER: Yes. So this was, I think, in May 2011.

FURNESS SC: It was.

WITNESS SKINNER: So she was reviewed by a cardiology registrar in Westmead "Following five episodes" - this is a summary of what he says in

that letter:

> "Following five episodes of chest pain over the previous year. The chest pain was described as atypical for angina and occurred at times of stress". Her doctor describes a normal 12 lead ECG, a normal exercise test with no ECG changes, diagnostic of ischaemia".

I haven't actually been able to see those ECGs, I don't know whether they still exist, but they were reported as normal by Dr Andriani who was the registrar.

FURNESS SC: The reference to her having had an exercise stress test, what does that involve ordinarily?

WITNESS SKINNER: So the - a 12 lead ECG is put on, the electrical ECG is for recording a normal ECG and then the patient is asked to walk on a treadmill and the ECG is recorded throughout the test and for a period of recovery after the test. If you have evidence of coronary artery disease where the heart muscle is getting short of oxygen or ischemic, then that ECG shows typical changes, such as the ST segment changes I mentioned earlier. So there, there were no diagnostic changes of ischaemia. I think one of the important things about this is that they - the doctor does not comment on any heart rhythm disturbance.

I think later on we're going to be talking about this condition CPVT, catecholamine-induced polymorphic ventricular tachycardia. So catecholamines are adrenaline. Adrenaline is released during exercise and if she had CPVT then I think we would have expected abnormal rhythm, ventricular extra beats during the exercise test and although I think we might expect that a Registrar might miss subtle changes of this or that, I don't think they would miss significant ventricular tachycardia during an exercise test.

FURNESS SC: Thank you. I notice, the time, your Honour.

JUDICIAL OFFICER: Yes, we'll take an adjournment for 20 minutes. Professor Skinner, I'm not sure what your situation is there, but you can go away for 20 minutes and come back again.

WITNESS SKINNER: Will do.

JUDICIAL OFFICER: Go and have morning tea, thank you.

SHORT ADJOURNMENT

JUDICIAL OFFICER: Yes, Ms Furness.

FURNESS SC: Thank you, your Honour. Professor Skinner, we're up to tab 47 of the tender bundle. That's an emergency response form dated 24 April 2014, do you see that?

WITNESS SKINNER: Yes.

FURNESS SC: The initial assessment is "Collapsed but responding" and there is an incident history which precedes it.

WITNESS SKINNER: Yes.

FURNESS SC: Do you draw any conclusions from that?

WITNESS SKINNER: Really difficult. This is the sort of thing where really it would be nice to have the patient in front of you and take more detail. Goodness me, "collapsed but responding" could mean an awful lot, couldn't it? The fact that she had back pain is a potential cause of a faint of course but really without more entry I'm not sure that I can take much from that.

FURNESS SC: Thank you. Perhaps if we can turn to the next tab, 48. These are progress notes for 23 June 2017 for Ms Folbigg. Do you see that?

WITNESS SKINNER: Yes.

FURNESS SC: You had a look at these notes, I think, and referred to them in your report.

WITNESS SKINNER: Yes.

FURNESS SC: Do you want to take us through them?

WITNESS SKINNER: Yes, sure. The clinical notes reveal that Kathleen turned white whilst in the bathroom and grabbed the bar of the toilet, it states, and slowly turned white and began to collapse. From reading through the general comments, she didn't injure herself but was found to be drowsy and pale and "actively vomiting" was the expression that's used. A note was made that during her medical evaluation her heart rate rose from 67 to 113 on standing and she felt washed out and thirsty and she was encouraged to drink more.

My take on this was with the presence of nausea and vomiting, thirst, and the very marked rise in heart rate on standing, all point once again to a decreased intravascular volume, dehydration, and I felt this was most likely a common faint or a near faint although she didn't completely lose consciousness, so we would call this a presyncope rather than syncope.

FURNESS SC: Putting together all of the various incidents I've taken you to this morning, does that alter the view that you formed in respect of the most recent being in 2017?

WITNESS SKINNER: No. I mean, I think it's important to state that all of this is very much a poor second to taking a history from the patient themselves and there's nothing - I'm hopeful that in the evaluation in a week or two that the cardiologist will take a very clear history of these events because that's really pivotal but everything I've seen so far, albeit lacking in detail on occasion,

really points to somebody who tends to nearly faint or faint in the presence of a vomiting illness or being dehydrated and during pregnancy so I think these don't sound like arrhythmic collapses. They don't sound like it's a primary cardiac problem. It's a circulatory problem, situational, common fainting, that's the general picture I'm getting.

FURNESS SC: Thank you. Can I have tab 56 on the screen. Now we're coming to 2018, Professor, and you refer to this towards the bottom of page 7 of your report.

WITNESS SKINNER: Yes. This is a good quality ECG which has been done as part of the current investigation. Her heart rate is 64 beats a minute. It's a normal sinus rhythm and the automated QTc at the top left there you can see - this is quite important because long QTc would have to be one of those rare conditions which could cause this but that's the top right there, yeah. See the QTc at the bottom there is 422 so the machine is telling us that the automated heart rate corrected QT interval is 422, normal being up to about 470 in an adult female. So this is plum in the normal range and then when I went and measured it myself manually - because sometimes these machines can get it wrong - my measurement is between 0.40 and 0.42 or 400 and 420 milliseconds, so again, plum in the normal range.

FURNESS SC: What's the difference between QT and QTc?

WITNESS SKINNER: QT is the absolute measurement and the QTc is the heart rate correction so, generally speaking, as your heart rate goes up the QT interval goes down and so you have to make an allowance for the actual heart rate at the time.

FURNESS SC: So tell me again the conclusions that you draw from those figures in 2018?

WITNESS SKINNER: I think this is a normal ECG with no features to suggest conduction system disease, Long QT syndrome. There's no Brugada signature, there's no abnormality of repolarisation; so I think it's normal with some important negative findings.

FURNESS SC: What are the negative findings?

WITNESS SKINNER: The absence of Brugada signature, the absence of a Long QT interval.

FURNESS SC: You mentioned earlier CPVT.

WITNESS SKINNER: Yes.

FURNESS SC: Is that something that this result is relevant to?

WITNESS SKINNER: No. CPVT is the catecholaminergic polymorphic VT so they often have normal resting ECGs. The presence of a normal 12-lead ECG

does not exclude that condition.

FURNESS SC: What about IVT?

WITNESS SKINNER: IVT?

FURNESS SC: You're going to ask me to tell you what it is, aren't you? We'll come back to IVT. I have it somewhere. I will come back to IVT.

WITNESS SKINNER: Right.

FURNESS SC: If we can then turn to tab 57, that's a chest X-ray report?

WITNESS SKINNER: Yes.

FURNESS SC: What does that tell you?

WITNESS SKINNER: Of course, I haven't seen the X-ray but it's a normal chest X-ray. The lungs and pleural spaces are clear. There are no masses and the cardiac size and contour are within normal limits. What this tells us, this is a more crude way of looking at the heart size, the echocardiogram that was done earlier is much better but this is consistent with a normal heart size. It's not enlarged as it often is in a cardiomyopathy.

FURNESS SC: Thank you. Idiopathic ventricular tachycardia.

WITNESS SKINNER: Right. I think idiopathic ventricular fibrillation is probably the thing that's going to come up in later discussions. Again, the resting ECG can be completely normal in that condition if it's ventricular fibrillation. "Idiopathic" just means unknown. It just means that nobody knows why somebody has got a ventricular tachycardia or nobody knows why somebody has got ventricular fibrillation. It's a medical term meaning unexplained.

FURNESS SC: Turning over to tab 58 this is even more recently, February this year, 22 February. That's an echocardiography report.

WITNESS SKINNER: Yes.

FURNESS SC: Do you see that? What does that tell you?

WITNESS SKINNER: This echocardiogram is reported as normal by Dr Mikhail Altman at Westmead Hospital. Important negative findings include a normal chamber size, normal left ventricular septal and free wall thickness and normal left ventricular function. Again, what this is telling us is that the heart muscle is neither abnormally thin nor abnormally thick and it is functioning normally.

FURNESS SC: You make comment in your report on page 8 on the cardiac phenotype in Kathleen.

WITNESS SKINNER: Yes.

FURNESS SC: Can you tell us what comments you have in respect of that?

WITNESS SKINNER: What I would say is that a complete investigation by today's standards would include tests looking for occult disease so disease which is not overt on the simple tests. That would include taking a detailed cardiac history which we're going to get; an exercise test which we're going to get; and a 24-hour Holter which is a 24-hour ECG recording which I hope we're going to get. We might want to do extended tests like a cardiac magnetic resonance imaging test and drug challenge tests. Certainly if somebody presented now with four deaths in the family they would have the whole book thrown at them.

However, I think we can make some useful conclusions. Kathleen is now over 50 years of age and this is 50 years over which an inherited heart condition can present itself and signs on cardiac tests can present themselves. So conditions which cause sudden deaths such as hypertrophic or dilated cardiomyopathy, they tend to progress over time and if she was going to develop these conditions I think by 50 we could reasonably expect some clinical signs - an abnormal ECG or an echocardiogram by now. She has got no features of these cardiomyopathies. Regarding cardiac ion channelopathies you can't see these; even when the heart is taken out of the body there's nothing to see. It's a microscopic thing. But she has not had a cardiac arrest in her 50 years. The syncopal episodes that I've reviewed would be consistent with situational or vasovagal syncope rather than arrhythmic syncope. The ECGs show no features of Brugada syndrome or Long QT syndrome nor do they show any sign of conduction system disease.

So I think we can make some general conclusions. As a specialist in Long QT syndrome I don't think she has Long QT syndrome and I don't think she has got any ECG features to suggest she's a gene carrier for it either on her two 12-lead ECGs. So I don't think it's likely she has got Long QT syndrome.

FURNESS SC: You say that you think it's unlikely that she carries a pathogenic Long QT gene. What does that mean?

WITNESS SKINNER: Long QT syndrome is a familial autosomal dominant condition and it has variable penetrance which means that some people who carry the gene can have a really bad form of it and have overt changes on their ECG but about a third of people who actually carry a potentially pathogenic change in their gene can have a normal QT interval some of the time. But despite that, Kathleen's QT intervals are at the sort of shorter end of the normal range and the shape of the T-wave, the way the ECG looks, is normal. Often even when people if they're gene carriers their QT interval might be normal but there will be subtle changes in the T-wave which gives a clue to the presence of occult disease. Of course, putting that together with the fact that she has got to 50 without having a cardiac arrest adds to that.

FURNESS SC: Thank you, Professor. Turning to you, Dr Colley, you have

provided your views on medical genetic advances in that document which I tendered earlier. Would you be able firstly to define for us some of the key terms that we need to understand when considering the advances that have been made, and how that is going to be applied over the next couple of days in evidence? If you could start with DNA.

WITNESS COLLEY: Alright. That's probably a good place to start. DNA is the molecule which encodes or is written in the genetic instructions. Each genetic instruction is a gene. This DNA molecule is inside all the cells of our body in the nucleus or the centre of the cell, the office or the hub of the cell. The DNA is a double-stranded molecule and people would be aware of that double helix picture that people see. Down the sides are phosphate and sugars that are bound together and the rungs of the ladder are the bases or nuclear bases which are purines and pyrimidines and they are joined together with hydrogen bonds so you have the ladder sides, you have the rungs and then it is coiled. So it's a double helix, it's helical and then it is also coiled around proteins called histones and that allows a lot of DNA to be tightly coiled and protected and a large amount of DNA into a small space.

The DNA is coiled into strands that are called chromosomes and we heard about chromosomes earlier. There are 23 pairs of chromosomes in the human genotype. They're numbered from the largest, number 1 down to number 22 which are the smallest and then the sex chromosomes are the 23rd pair, two Xs in a female and one X and one Y in a male because there clearly has to be some different instructions to make the differences between the genders.

These are inside all the cells of our body. They are important because those genes that are encoded in that DNA are the instructions for what we look like, how we grow from being a single cell up to a baby, up to an adult, how our bodies work, how they function, how the different cells do the jobs in our body that they need to do as well as obviously our reproduction. That I think is probably my explanation of DNA. If people want anything more?

FURNESS SC: What can we do with DNA today that we couldn't do with DNA in 2003?

WITNESS COLLEY: We can study it much closer. So we know that as you've mentioned there's the exome which is the code, they're the genes that we - that code for particular proteins. We can study those and we can look at the DNA little piece by little piece. So the phosphate, sugar and nuclear base is one nucleotide and we look at - we can look at every step. So we're looking at every word in the instruction and we can look at the spaces between the words, which are called introns and we can look and see whether there's any spelling mistakes there at all. We also do a quantitative measurement and look and see if there's anything missing, any little bits that have gone missing or anything duplicated or extra. So we can look at quantity as well as quality of the DNA, but we really couldn't do in any way back then.

FURNESS SC: What is a variant?

WITNESS COLLEY: A variant is a change. It's - we usually mean a change in one of the nuclear bases and there are four bases, adenine, thymine, guanine and cytosine. Those ATCG are the sort of - the code that makes the spelling of the words that make up the sentence. A variant means that we've changed one base to another. Now many times in fact a change doesn't make any difference at all to the outcome of the word or to the meaning of the word. So in other words, the protein may not change just because there's a variant.

And that's why this way of saying the, the - American guidelines saying that we look at each variant and say, is it definitely pathogenic which would mean it would alter the resultant protein in such a way that would cause a change to the cell. Is it likely pathogenic? Is it a variant in the middle that we really don't know if it's going to cause a change in the protein that would lead to something different in the cell and thus the organism? Is it likely benign or definitely benign? And by "benign" we mean harmless. It's not going to cause any change.

FURNESS SC: Just because a person has a variant does that mean that they've got a disease that's going to cause them harm?

WITNESS COLLEY: No, in fact all of us have variants, all of us have thousands of variants in our DNA. And the vast, vast majority of variants are not going to cause us any disease or harm at all. And most of us of course we're not going to have our genome sequenced and we'll go through life not knowing what those variants are and it doesn't matter.

FURNESS SC: What happens when the whole genome is sequenced in a person?

WITNESS COLLEY: Well, you - from my point of view, from the clinician's point of view I obviously talk to the people about what that might mean, what that will mean for them, what sample is going to be collected, that we're going to read the genome and we're going to look at variants and there's going to be lots of variants--

FURNESS SC: So is the purpose to find variants? Is that why you do sequencing?

WITNESS COLLEY: I would only - as a clinician I do sequencing of people where there is a phenotype, there's a disease or a condition and I'm trying to find out whether there is a genetic change which is causing that phenotype that I'm seeing. So my purpose is yes, to find a change that would be considered causative to the phenotype. Sometimes doctors do send us patients, say a paediatrician might send along a child to say, I want to rule out genetic conditions. That's not the way it works. We don't do gene sequencing to prove a person is normal. A person is normal is normal. We have patients who have some abnormality or difference about them and we'll do genotype to try and find the genetic cause and finding--

FURNESS SC: What is a genotype?

WITNESS COLLEY: A genotype is the genetic makeup, the - he looked - the genotype is all the genes in the - is the, the reading of those genes. The genes - looking at any variance within those genes.

FURNESS SC: How does a phenotype and a genotype sit together?

WITNESS COLLEY: Well, the phenotype is the observable or characteristics that we mentioned before and those observable characteristics can be due to changes in the genes, either quantity or quality. They - the phenotype could also be caused by environmental conditions as well. Sometimes it's a combination of both the genotype and the environment and sometimes it's the interplay of the genetic predisposition and the environmental effects. So the genotype is what is happening in the nucleus or the centre of our cells, which is in sometimes related to the phenotype which we're seeing in a person.

FURNESS SC: So is it the case that today the advances since 2003 in your area is in the ability to provide Whole Exome Sequencing ("WES") and Whole Genome Sequencing ("WGS") on DNA?

WITNESS COLLEY: Yes, that is a very main increase in our ability to provide genetic information. I'd say that the - quantifying the DNA, looking for pieces missing or little bits extra, has also improved tremendously in that time.

FURNESS SC: And in relation to the work that has been done for this Inquiry, has that involved any of the two matters you've just referred to?

WITNESS COLLEY: Yes, both of them. So there was both quantity and quantitative measurements done on some of the samples and also the genotyping or the reading of the code on the samples that you mentioned earlier by Whole Genome Sequencing.

FURNESS SC: Is it the case from what you've said that the sequencing provides us with a knowledge of variants and then there is a separate exercise to determine whether those variants matter in the sense that they are causative of some disease and then it's a question of looking at the phenotype of the person in respect of the disease that may have been caused by the variant?

WITNESS COLLEY: Yes, absolutely. So as we've heard you mentioned earlier that it's a multifaceted approach, we take into account the phenotype of the patients or the people we're seeing and the genotypes and that - those are looked at separately and then considered together. So it, it is important, because there are so many variants, just having a variant doesn't - even in a gene that we know can be harmful if the variant was pathogenic, but having a variant in itself does not mean a person has a disease.

FURNESS SC: Just remind us, what is the step that needs to be taken after identifying a variant?

WITNESS COLLEY: Well, the most important step is what my colleagues here in molecular genetics do is that they look at that variant and say, this change in this gene at the DNA level, what would it change when it comes to the resultant protein that this gene is supposed to be making. How would the protein change? Maybe there would be no protein, maybe an abnormal protein, maybe a protein that does a job, but not very well. So they'd have a look at that side and then they'd say, well if this person had an abnormal protein or a missing protein, what is known about the phenotype when that occurs? What is in the medical literature that we can use? And then we'd say, well okay, let's look at the phenotype of the patient or patients we've got here and is there a correlation in any way?

FURNESS SC: Thank you. Dr Buckley, do you want to add to that general description by Dr Colley?

WITNESS BUCKLEY: No, I think it's a very nice summary description, exactly.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Well, perhaps just to extend slightly to say that the, the idea of, of a variant implies that there is some sort of benchmark against which that's compared and although it's true there is what is called a reference sequence, that's something of an artificial construct, all of us have our own genome that is unique to us and so a variant is really just against a reference, which in some cases is a little bit arbitrary, because there may be, there may be different choices to what should be the, the reference. And to reinforce that point that an understanding of the phenotype is a very important part of our ability to classify whether a variant is potentially disease-causing or not.

FURNESS SC: Thank you. Coming now to the Inquiry process, each of you were engaged by the Inquiry to advise initially on the process that could or should be followed in respect of genetic testing of initially thought to be the four children, is that right? You'll just need to answer each of you?

WITNESS COLLEY: Yes.

WITNESS KIRK: Yes.

WITNESS BUCKLEY: Yes.

FURNESS SC: And I think three meetings were held at which each of you were either present or at least present for one or more of them, that's right?

WITNESS COLLEY: Yes.

WITNESS BUCKLEY: That's correct.

WITNESS KIRK: Yes.

FURNESS SC: And Professor Skinner, you were part of this as well? You'll

have to unmute yourself.

WITNESS SKINNER: Yes, that's right.

FURNESS SC: One of the principal purposes of the meetings was to agree amongst yourselves, together with Professor Cook and Professor Vinuesa the process which was to be followed, is that right?

WITNESS KIRK: Yes, it's--

FURNESS SC: Does anyone disagree with that? No. And that process included looking at the available samples, yes?

WITNESS BUCKLEY: Yes.

WITNESS COLLEY: Yes.

WITNESS KIRK: Yes.

FURNESS SC: Looking at the facilities that might be available to do the work needed?

WITNESS COLLEY: Yes.

FURNESS SC: Applying the ACMG guidelines as to pathogenicity?

WITNESS COLLEY: Yes.

WITNESS KIRK: Yes.

WITNESS BUCKLEY: Yes.

FURNESS SC: And looking at it from a hypothesis free perspective?

WITNESS COLLEY: Yes.

WITNESS BUCKLEY: Yes.

FURNESS SC: Is that right? Are there any other areas of agreement that arose out of those three meetings that determine the way in which you all carried out your work? Perhaps starting with you, Dr Colley?

WITNESS COLLEY: No, I think that's it. We did - although I think it's very important we started with hypothesis free, which means we're looking at all possibilities, all genes that could cause catastrophic events, or infant demise. Following on from that though there was a candidate gene list prepared by various specialties, specialists that we also agreed to go back and look at in more detail or re-analyse.

FURNESS SC: Dr Buckley, are there any areas that have, have been missed

in terms of the agreement as a result of the various consultation meetings?

WITNESS BUCKLEY: I think the only other point I would make is that we agreed that the two laboratory processes would be independent of each other and that they would necessarily generate slightly different results. Though we expected the majority of data to be overlapping, there were to be some differences between the laboratories that would be expected and that would be a perfectly normal part of the processes that we undertook.

FURNESS SC: Professor Kirk, do you have anything to add?

WITNESS KIRK: I guess the only other thing, you may have already covered it actually, is that there was an agreement that as far as possible the testing should be done in accredited laboratories.

FURNESS SC: Yes, we'll come to that in a bit more detail. Professor Skinner, is there anything that's been missed?

WITNESS SKINNER: No, thank you.

FURNESS SC: All right. Can we first come to the samples that were available to you and if we can have up tab 61? Do each of you have it in front of you in hard copy? Here it is. So these were the specimens that were available and we can see that with Caleb it was the newborn screening test, which effectively means a blood spot, is that right?

WITNESS BUCKLEY: That's correct, yes.

FURNESS SC: And then with Sarah, the same blood spot was available, together with various other matters that are addressed in that column? Yes?

WITNESS BUCKLEY: Yes, that's correct.

FURNESS SC: And then moving down to Patrick, if that's further down the page - I'm sorry, Laura is next. In addition to the blood spot there were also tissues that were available?

WITNESS BUCKLEY: That's correct.

FURNESS SC: And with Patrick, it was the same, there were tissues as well as blood available?

WITNESS BUCKLEY: Yes.

FURNESS SC: I think additionally with Patrick, if we can have tab 64, there were some other tissues that were available and do you see they're set out there in the email? That's right? So the case is all of those tissues or blood samples were available to you to consider what testing could and should be carried out, is that right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: What you also had was Kathleen's DNA. Can I ask you first, Dr Buckley, having Kathleen's DNA provided a benefit, advantage, disadvantage to the work you had, was neutral?

WITNESS BUCKLEY: Yes, it provided a clear advantage that we would be able to then determine whether a variant was present just in a child or was - in fact had been inherited from that child's mother, Kathleen and you could then draw clinical - some - you could use information about clinical state in Kathleen to help inform us about the interpretation of that information.

FURNESS SC: The amendments that were made to your joint report earlier this morning, to add the fact that Kathleen was alive and apparently well, affected your classification of various variants, is that right?

WITNESS BUCKLEY: Yes, two variants, yes.

FURNESS SC: Does anyone want to say anything else about having Kathleen's DNA available? No? In terms of Craig, you weren't provided with any DNA sample from him, that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: What effect, Dr Buckley, did that have on the work you could do?

WITNESS BUCKLEY: Rather surprisingly it didn't have much effect. We did not identify any variant in the children that we were concerned about that appeared to have been inherited from Craig, and the interpretation did not hinge on his clinical state.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yeah, I'd agree with that. If, if upfront we had had the option, we would certainly have preferred to do that because there is a possibility of a mechanism for which interpretation would require both parents. But in the end it didn't make any difference.

FURNESS SC: Dr Colley?

WITNESS COLLEY: Yes, I'd agree with what's being said, and I was pleased that I had had an opportunity to meet him in person, so I did know that he was of normal statute, normal intelligence and normal appearance.

FURNESS SC: Did the absence of Craig and any broader members of the Folbigg family put you in a position where there was uncertainty as to the phenotypes so as to render the work you have done less useful? Dr Colley?

WITNESS COLLEY: No, I don't think so. I think if we had found a possibly

pathogenic or likely pathogenic variant, that we wanted to trace or we say segregate through the family, then it would have been a disadvantage not to have DNA from other family members. But as such, as you've heard we didn't actually identify such a variant, so therefore we didn't need the DNA from the other family members.

FURNESS SC: If you had that DNA, let's limit it to Craig for the moment, would have that have enabled you to identify variants that you haven't otherwise identified?

WITNESS COLLEY: No, I don't believe so.

FURNESS SC: They may have helped you to identify variants in Craig but not in the children?

WITNESS COLLEY: Yes.

FURNESS SC: Dr Buckley?

WITNESS BUCKLEY: Yes, that's correct. We - each analysis was performed independently of each other. We should have seen all the variants that were available, and that the observation of a variant in a child would not have been dependent on whether we'd seen it in Craig or not.

FURNESS SC: Professor Kirk?

WITNESS KIRK: I agree with that.

FURNESS SC: Can I turn to the selection of facilities and the tests, and you deal with that in your report on page 4. In one of those early meetings there was a discussion as to which facility or facilities might perform the testing, that's right?

WITNESS KIRK: That's correct, the meeting of December 10 if I remember correctly.

FURNESS SC: Dr Buckley, on page 4 you indicate there that Professor Vinuesa provided information about the Victorian Clinical Genetics Service in Melbourne, that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: I think there was some contact with that service and ultimately it was agreed that it would perform what tests?

WITNESS BUCKLEY: If I might, I might start by saying that the first laboratory of choice was the Australian Genome Research Facility because--

FURNESS SC: Can you just say that again?

WITNESS BUCKLEY: The AGRF, the Australian Genome Research Facility. The reason for that was because it was already at that time accredited for performing Whole Genome Sequencing, whereas at the time that we had the discussion the Victorian Clinical Genetics Service was yet to be accredited for that service.

FURNESS SC: What does accreditation give one?

WITNESS BUCKLEY: So what accreditation means is that the laboratory has prepared a group of evidentiary data that supports that they are able to perform the testing that they claim to be able to support, that that data has been independently assessed by an expert, and both the information relating to the conduct of the test, and information relating to how the laboratory organises itself have been assessed through the National Association of Testing Authorities, NATA, who in combination with the Royal College of Pathologists of Australasia, then accredit the facility as providing a - to essentially a standard of care.

FURNESS SC: So the accredited facility, was that used or not used?

WITNESS BUCKLEY: The accredited facility, the AGRF, was used to examine the tissue samples from, from Patrick, from Sarah and from Kathleen. They were not accredited to perform testing from a blood swab and so we did not ask them to do that, because that fell outside of scope.

FURNESS SC: So who looked at the blood spots?

WITNESS BUCKLEY: So on the advice of Professor Vinuesa we were aware that the Victorian Clinical Genetics Service had actually gone through the process of accumulating all the validation data on blood spot testing, but had yet to be assessed by NATA and in discussion at that meeting with Professor Cook and Professor Vinuesa we thought that since there was only blood spot material available from Caleb and from Laura, that it would be better to have some evidence performed in a laboratory which itself was accredited, where they've been through the validation process but were pending accreditation for genomic testing on blood spot cards, rather than to have no evidence at all. And so we chose to send two samples to the VCGS and three samples to the AGRF.

FURNESS SC: Then there was also chromosomal microarray testing. How did that come about?

WITNESS BUCKLEY: So as Dr Colley has indicated, copy number variation, the amount of DNA present, or whether it's duplicated or deleted, is also an important contributor to genetic disease, and there was sufficient DNA available from the tissue samples at the AGRF that they were also able to perform on those same samples a technology which we call copy variant, copy number detention, by chromosomal microarray.

FURNESS SC: Was there any testing that you wanted to do that you couldn't

do because of the quality of the samples?

WITNESS BUCKLEY: No, I don't think so. We, we were - yes, actually, sorry, I beg your pardon, there was. So we had initially intended to do Whole Genome Sequencing on Laura's sample on her blood spot, which - to match the Whole Genome Sequencing performed on Caleb's sample on his blood spot, both at the VCGS, but it was found after that process was started that Laura's blood spot sample had been heavily colonised by bacteria and so the Whole Genome Sequencing, the genomes that were got out of that blood spot were predominantly microbial genomes.

So on the advice of the Victorian Clinical Genetics Service's director, Sebastian Lunke, we proceeded to do Whole Exome Sequencing on that one sample, because that process involves an enrichment step where you can enrich for the human DNA component to the exclusion of the microbial data. Bearing in mind that we were assessing all of these variants together as a family, so although we would expect there might be some variation in quality of DNA, we could use the - we could leverage the information available from the entire family, barring Craig, to help determine whether a variant was truly present or not, or truly absent.

FURNESS SC: Professor Kirk, do you have anything to add to that?

WITNESS KIRK: No, I don't think so, no.

FURNESS SC: Is it the case that there was no test that was available that you wished to use in respect of the DNA that you couldn't use?

WITNESS BUCKLEY: No, I was satisfied at the end that we - given the age of the material - that we had sufficient material to proceed with Whole Genome Sequencing and the Whole Exome Sequencing in the residual case was adequate.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yeah, I mean I think it's remarkable that it was possible to perform testing of this type on these samples, and the resulting data quality was good, yeah.

FURNESS SC: Because of the age of the samples?

WITNESS KIRK: Yeah. I don't know that something like this has been attempted before, at least not in this kind of context, and the outcome was that we got very high quality data that was able to be interpreted.

FURNESS SC: Dr Colley?

WITNESS COLLEY: Nothing more to add, just agree.

FURNESS SC: Can I come to the clinical assumptions that you made in

carrying out your work. Firstly, again on page 4, I'm not sure which of you wishes to answer this, but let me ask the question and then you can decide who wishes to answer it. The first clinical assumption is in relation to the frequency of SUDI, and you say that the incidence of SUDI in Australia in 2016 was 1 to 3,300, with SIDS representing 70 to 80% of all SUDI. So you've said that in your report, but the question is firstly, why did you take that into account?

WITNESS BUCKLEY: Because it's significant in - so the frequency of the disease in a population, is one parameter that we, we look at. Each allele, each variant--

FURNESS SC: So what is an allele?

WITNESS BUCKLEY: So I'll come back to that, I'll use the word allele and variant interchangeably. An allele is just an identifiably different form of a particular gene, and the cause of those differences are variants. So wherever I use the word variant and/or allele I will generally mean that they are equivalent. So variants come with frequencies in the population in the main, and that if you have a variant which is present in every single person in this room, as an example, then you would say that that could not explain a rare disease such as sudden undiagnosed - sorry, sudden unexplained death in infancy, because that's only got a frequency of one in 3,000, if it's simply too high a frequency in the population for it to be a plausible cause of that death. So we used the information about frequency to help sort the very, very, very large number of variants that every single one of us has into a management number of variants for analysis.

FURNESS SC: If the data, that is by way of published literature in the population databases and the like available to you said that a variant was in fewer than one in 3,000. Did you put that to one side?

WITNESS BUCKLEY: No, it was - we used it in the - we, we studied those variants, because SUDI itself is an aggregation of different causes of death one assumes with multiple potential causes.

FURNESS SC: So if it was more than one in 3,000 you'd put it to one side?

WITNESS BUCKLEY: Yes in fact, but we were, we were cautious. We, we really only excluded things if they were present at more than one in a thousand, so we set a reasonably, a reasonably generous cut-off so we didn't get any false negatives.

FURNESS SC: So your cut-off was one in a thousand notwithstanding the data you had about SUDI being effectively one in 3,000?

WITNESS BUCKLEY: Yes, we were overly - we were over cautious I think perhaps.

FURNESS SC: You then in your second paragraph refer to investigations of

the percentage of SUDI under one year can be attributed to monogenic causes, and then refer to various literature. Can you tell us what that means?

WITNESS BUCKLEY: So we're trying to understand what the, what the - if there was a genetic - Sudden Infant Death Syndrome, as I've just indicated is an aggregation of different causes. As far as we can tell we can identify somewhere in the range of two to 20% of causes can be attributed directly to monogenic genetic causes.

FURNESS SC: And that's from the published literature?

WITNESS BUCKLEY: That's from the published literature.

FURNESS SC: Which you set out in paragraph 2?

WITNESS BUCKLEY: Yeah. In the majority of cases of sudden unexplained death infancy a genetic cause is not found.

FURNESS SC: So given that a genetic cause is not found, did that affect the way in which you filtered or otherwise interpreted the variants available to you?

WITNESS BUCKLEY: No we didn't - that didn't affect the, the filtering steps, no.

FURNESS SC: Now the third paragraph, you say that any putative genetic condition that resulted in four deaths in such young siblings would by definition have to be unusually severe. How did you take that into account?

WITNESS BUCKLEY: I've taken this comment particularly offered by Professor Kirk, so I'd ask if Professor Kirk could address to that question please.

WITNESS KIRK: Sure. So addressing that paragraph, there are many genetic conditions which vary in severity and one of the ways that that can manifest itself is in relation to age of onset, so that at the milder end of a condition you might have onset - there are some examples where the range is from a lethal condition in infancy through to a very mild condition in late adulthood, and what we're saying here is that if you're talking about something that can cause multiple deaths in very young children then by definition you're talking about a severe condition. You cannot be talking about something that is at the milder end of the spectrum.

FURNESS SC: Thank you. Is there anything that you wanted to say Dr Colley about the clinical assumptions?

WITNESS COLLEY: No, I certainly agree with all those.

FURNESS SC: Professor Skinner? No? Can we then turn to the variant analysis and that's at page 6 of your report.

JUDICIAL OFFICER: I'm just wondering whether Professor Kirk wanted to add something?

FURNESS SC: Did you want to add something Professor Kirk?

WITNESS KIRK: 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 two 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.

FURNESS SC: Smaller than one in 1,000?

WITNESS KIRK: Smaller than one in 3,300, so -

FURNESS SC: I understand that, but Dr Buckley said about one in 1,000. Is that about right?

WITNESS KIRK: That's the number that we used for filtering purposes and it's a very conservative number.

FURNESS SC: Coming to the variant analysis, it might be useful at this stage to have on the screen a diagram that Dr Buckley has provided to assist in understanding this. Can we have the funnel on the screen? Dr Buckley, can you take us through the funnel?

WITNESS BUCKLEY: As we've alluded to frequently, every single one of us has a huge number of variants. Every single person in this room has got three million variances from human reference sequence. So the likelihood of any one variant being associated with a severe disease is remarkably low, less than one in three million one would say. So in order to take this very large universe of variants and reduce the question to a manageable size, which ones of these can be associated with disease, we go through a process of variant filtering and prioritisation.

Firstly, out of that very large number of three million variants we choose to focus on those that lie within genes that are known to code for proteins, because they are the effector - they are the things that do things with themselves, the building blocks of cells in essence. We also - so that reduces three million down to just 55,000 because as we have - as counsel made reference to in her opening comments, the exome, the protein coding region of the genome, is only 1% to 2% of the entire total. So we then focus further on those still very large number of variants within genes and we exclude those which are at high frequency in the population, and we exclude any with a CAD score of less than ten.

A CAD score being a general method of prioritisation of variants based on some software predictions. Both the Sydney laboratory and the Canberra laboratory, they both independently and somewhat to my surprise, used the

same filtering mechanism, the CAD score. We have a slightly different threshold. Ours was again, just very slightly, more conservative than the Canberra laboratory, so we would end up with a, a slightly number of variants to deal with than they did. So that brought us down from 55,000 down to 1,600. In amongst that 1,600 - sorry, 1,677, and here I use particular data which were taken from the data from Patrick Folbigg's sample, among those 1,677 we were then able to exclude variants that although they were known to be in genes--

FURNESS SC: Let me stop you there. In-house database. What does that mean?

WITNESS BUCKLEY: So the laboratory I, I am the Clinical Director of, also uses the Australian Genome Research Facility for some of its testing, so we've sent quite a large number of samples to the AGRF for other purposes. We've never sent a sample to the AGRF for the purpose of identifying the cause of SUDI. So we have a database from their exome sequencing information which tells us how frequent particular variants are in the HRF data set. That's important because every laboratory's sequencing procedure is subject to its own artefacts, its own site specific or laboratory specific changes. So we were then able to exclude from the 1,677 variants which were present that we knew of that were in the AGRF data routinely across many samples that we had sent away for other purposes. We also excluded some variants that were homozygous in the population.

FURNESS SC: What does that mean?

WITNESS BUCKLEY: It means that we - every one of us inherits a copy of each chromosome from our mother and a different copy from our father. For homozygous it means that a variant is present on the paternally inherited allele and the maternally inherited allele. Now, if you've got a, a - if you've got a variant which can cause a disease, having essentially twice the dose of that is perceived as being a, a - it's, it's very likely to be a disadvantageous outcome. And so, for things which are present in homozygous state, at high frequency in the population, in a disease which is severe and paediatric onset, one can be - they can be routinely excluded, and so we exclude them.

FURNESS SC: Do you understand that the Canberra laboratory excluded in the same manner that you did?

WITNESS BUCKLEY: Yeah, I, I, I think it's remarkable that although these two systems have been developed completely independently of each other, we have essentially chosen the exact same process of filtering and prioritisation. There, there are minor differences, but it's remarkable how consistent they are between two laboratories which have developed them completely independently, I think.

FURNESS SC: So, you then come down to excluding variants that were sequencing artefacts?

WITNESS BUCKLEY: Yes, so, once I got to the candidate group of 681 in Patrick, I then visually inspected every single one of those to determine whether they had - until this point, I had not done any filtering based on quality, because I didn't want to over-filter and throw out a, a sequence that, that looks poor, but could still be pathogenic. So, at this point, I went for sequences where we had good evidence that they were sequencing artefacts, based on a number of different appearances on - in the, what we call the integrated genome viewer, IGV, trace of that. So that - because we had not done any filtering on quality to this point, I was able to deprioritise a further two thirds. And so, we were left with a group, and when we aggregated across the five individuals who we had samples for, we were left with a group of 279 variants that were present in at least an individual in this five-member family.

FURNESS SC: So, it just had to be present in one?

WITNESS BUCKLEY: Sorry? Yes, sorry, I'm - it - I'm, I'm going from here to - from Patrick to, to generalising--

FURNESS SC: Yes.

WITNESS BUCKLEY: --I beg your pardon. So--

FURNESS SC: No, I--

WITNESS BUCKLEY: --Patrick's data relate to the green and the light blue parts of this. So, we're left with 279, that has to be present in at least one and they have to be high confidence variants. And then, among those, both Professor Kirk and Dr Colley independently, plus myself, reviewed every single one of those variants to determine if there was a known disease association and whether, if there was a known disease association, was that relevant to the phenotype under investigation in this family. And that really brought us down to - quite remarkably. We were able, of our starting putative three million samples - three million variants, we got down to about nine where we wanted to make sure that there were - we wanted to be reassured that we understood the characteristics of those and whether they were likely to be disease causing, or uncertain, or benign.

FURNESS SC: What was your source of information as to a known disease association?

WITNESS BUCKLEY: So, in general, we enquire - queried the medical literature and the particular mechanism we used to do that was the PubMed database. So, throughout my reports you will see - sorry, I beg your pardon, our report, you will see a reference to the, the abbreviation PMID, occasionally PMIDs for plural, meaning PubMed Identification Numbers, which is a unique identifier of a particular publication, which would then be relevant to, to part of the, the phenotype, or may not be.

FURNESS SC: Thank you. Professor Kirk, do you want to add to that?

WITNESS KIRK: I guess I'd make the point that, although Dr Buckley is right, that this is remarkable that we can do this, it's also true that there's nothing unusual about these variants. If we did the same exercise in anyone in the room, we would expect to come up with similar numbers.

WITNESS BUCKLEY: Yes.

FURNESS SC: Thank you. Dr Colley?

WITNESS COLLEY: No, that's fine.

WITNESS BUCKLEY: If, if I had to make one emphatic comment, that the presence of a variant does not imply disease, it just is a difference from, from the population state or the reference genome. So, that's--

FURNESS SC: So, that's why it was necessary to analyse whether the variant that the gene was located in had a known disease association?

WITNESS BUCKLEY: Yes.

FURNESS SC: Because without the known disease association, it just simply didn't matter?

WITNESS BUCKLEY: Well, yes. I mean, there's - it's, it's - it was impossible to interpret without a known disease association.

FURNESS SC: And then, having determined the known disease association - that is, where there was one - then you look at the clinical presentation or the clinical information or phenotype available about the individual, to see whether or not it might be relevant?

WITNESS BUCKLEY: Yes.

FURNESS SC: And that’s when you get down to the nine? Thank you.

WITNESS BUCKLEY: So, so, I think counsel made the statement again in her opening comments, that this is a single, multifaceted test. There is a very important part of it, which is the phenotyping of the patient, which happens in the clinic by the experienced physician - a cardiologist, neurologist, clinical geneticist. We generate part of the necessary information in the laboratory and then we tie those together. It is a single, multifaceted test across different domains, with different people providing different components of the information. But it's the totality of the data that makes the clinical diagnosis, not the laboratory information in isolation from the clinical information or vice versa.

FURNESS SC: Thank you. Your Honour, I tender the funnel, genomic variant--

EXHIBIT #AD FUNNEL TENDERED, ADMITTED WITHOUT OBJECTION

FURNESS SC: Thank you, your Honour. I note the time.

JUDICIAL OFFICER: Yes, we'll adjourn until 2 o'clock.

LUNCHEON ADJOURNMENT

FURNESS SC: Thank you, your Honour.

JUDICIAL OFFICER: Yes.

FURNESS SC: Can I turn now to tab 60, which is the American College of Medical Genetics and Genomics Standards and Guidelines. You might have a hard copy in front of you. Professor Skinner, you've got a copy of that? Tab 60? You're muted again.

WITNESS SKINNER: Yes, sorry, tab 16, I'll get that.

FURNESS SC: 60, sorry, six zero.

WITNESS SKINNER: 60?

FURNESS SC: Yes.

WITNESS SKINNER: Yep, I'll get to that.

FURNESS SC: I'll leave it to you to decide who to answer this, but these are the guidelines that were promulgated in 2015 by the American College of Medical Genetics and Genomics, that's right?

WITNESS BUCKLEY: That's correct.

WITNESS SKINNER: That's right.

FURNESS SC: And they are, I take it, well regarded in the area in which they work?

WITNESS BUCKLEY: Yes, they are. The American College of Medical Genetics and Genomics is one of the three or four largest national societies and has got a very, very high international reputation. Particularly for the quality of its standards and recommendations and guidelines.

FURNESS SC: Do you, in the work you do here in Australia, apply those standards and guidelines?

WITNESS BUCKLEY: Yes, we, we routinely apply these for the - to try and understand the pathogenicity of variants.

FURNESS SC: And in one of the various discussion meetings I referred to earlier, the fact of these guidelines came up for discussion, is that right?

WITNESS BUCKLEY: I'm sorry, could you say that again?

FURNESS SC: In one of the discussions between you and Professor Vinuesa and Professor Cook the guidelines came up as a topic for discussion as to their application to the exercise you carried out?

WITNESS BUCKLEY: Yes, that was correct and we decided--

FURNESS SC: And it was agreed that this was the appropriate guideline to follow?

WITNESS BUCKLEY: It is the best guideline and we were, we were agreed.

FURNESS SC: The disclaimer at the top of the document, it says that:

> "These standards and guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality services. Adherence is voluntary and doesn't necessarily assure a successful medical outcome."

How do you apply that disclaimer when you apply these standards?

WITNESS BUCKLEY: So the way I apply these is that as part of a set of evidence we use, we use clinical state, we use the evidence that emerges from this, plus any other evidence which is, is important. For example, these really only apply in the situation where, where the pathogenicity of a variant that's not yet been completely nailed down, if there is objective evidence from other studies that the pathogenicity of a variant is known, then you don't need to apply these. So it's, it's - it is one of a set of, of tools that we use to come to a, a medical conclusion.

FURNESS SC: Does anyone else want to add anything?

WITNESS KIRK: Yeah, I would just add that they are widely accepted in the community worldwide, but there have been some additional publications since then that adjust and modify the use of the tool somewhat and we are competent(as said) of those in making our assessments and also that they are a general set of guidelines that is intended to be broadly applicable, but there are some occasions when there are types of evidence that are not well captured by the guidelines and you have to be aware of that and able to apply that.

FURNESS SC: Did any of those occasions apply to the work you were doing for this Inquiry?

WITNESS KIRK: Yes, well, in relation to a variant in Patrick Folbigg in the IDS gene, there is some biochemical evidence which is not very clearly falling within the guidelines, but which is still important.

FURNESS SC: We'll come to the IDS gene.

WITNESS KIRK: Yes.

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

WITNESS COLLEY: No.

FURNESS SC: On page 406, the bottom of the second column, under the heading "General Considerations", there's reference to terminology and the recommendation is that a mutation, which is defined as a permanent change in the nucleotide sequence, whereas a polymorphism is defined as a variant with a frequency above 1,000 and then suggest that those terms shouldn't be used and instead recommend the five tier terminology that's on the top of 407, Dr Colley, you earlier gave some evidence about these terms. Can you tell us what they mean in the context of interpreting variants?

WITNESS COLLEY: Yes. Going back to the term "mutation", it's not one we generally use any more. Once upon a time people just thought about a variant in the sequence of a gene, called it a mutation and in, and in saying so there was a general assumption that it was going to cause a change in the protein product and therefore was going to change a phenotype. And now we know that that's not true. So we tend to steer away from that because I think it is a term that's more in the lay literature and not really a medically used term anymore. Polymorphism is just another word for change, again, another word for variation, but with the lay connotation that it's not going to cause a change in phenotype, that it's going to be harmless.

So those two terms "mutation" and "polymorphism" were taken - were both relating to a change in a gene sequence or a change in the DNA, which was thought to be either definitely going to cause a phenotype, an abnormal phenotype, or definitely not going to cause an abnormal phenotype mutation and polymorphism. That paper and these guidelines suggest we steer away from that and agree on five terms which gives more depth to the - what we mean by what the variation or the variant is going to cause. So we say pathogenic is when we believe the variant and we have evidence and evidence from the literature, evidence from various sources, that that change in the DNA changes the gene, changes the protein product in one of many ways, but would lead to a change at a cellular level and then at a phenotype, an observable level. The term "likely pathogenic", is where we--

FURNESS SC: Sorry, let me just stop you there. When you say "a change", is that a change with neutral consequences or perhaps harmful consequences?

WITNESS COLLEY: Sorry, I meant when there is a change in the DNA, where there's a variation, when we're talking about pathogenic, we're saying that it causes a harmful change or it causes - it doesn't - I guess it's not necessarily harmful. It's causing the change that we recognise as a phenotypic change. Now there's a lot of what some people say is perhaps harmful is a colloquial term. For some people in the deaf community, deafness isn't harmful, but we

would say it's a change from what is accepted as normal.

FURNESS SC: Thank you, so that's pathogenic. "Likely pathogenic"?

WITNESS COLLEY: Along the same lines, except we don't have the same level of evidence. Perhaps there isn't the same published literature. But when looking at the change in the DNA sequence and working through it, we believe from what we know about it, it would change the protein product and we would expect it or we think it's highly likely that it would cause a phenotypic change.

FURNESS SC: And we'll come to that, but there's criteria provided in these guidelines for looking at evidence that's very strong, strong, moderate and supporting?

WITNESS COLLEY: Yes.

FURNESS SC: "Uncertain significance", what does that mean?

WITNESS COLLEY: It means we don't know. We're sitting on the fence. Where sometimes is this, there just isn't the literature out there, there isn't enough known. Sometimes we've used these tools to try and get evidence and they've been conflicting. So we don't have clear evidence in one direction of pathogenic or benign.

FURNESS SC: The paragraph that we're referring to after those words are used, "Although these modifiers may not address all human phenotypes, they comprise a five tier system of classification for variants relevant to", is that Mendelian disease?

WITNESS COLLEY: Yes, Mendelian disease are those genes - are those diseases that are caused by single genes, as in, you know, Gregor Mendel and his peas and his colour of his pea flowers. So what we're meaning there is Mendelian disorders are caused by changes in a single gene which can be inherited in various fashions, dominant, recessive and X linked.

FURNESS SC: Thank you. Before I come to the databases, is there anything Professor Kirk or Dr Buckley you wished to add? Yes, Dr Buckley?

WITNESS BUCKLEY: So in understanding the concept variant of uncertain significance the likely pathogenic, pathogenic group and the likely benign, benign group, together account for only 20% of variation. The vast majority of variants are in the variants of uncertain significance category, but also the - it's, it's critical, I think, to understand that we have a very, very large number of variants. So the likelihood that any one variant is going to be pathogenic is remarkably low. Ed, would you care to expand a bit?

WITNESS KIRK: Yeah, so, so if you're looking at any one variant, the prior probability is that it's benign and so classification of something as a variant of uncertain significance. Most of the time if we had true knowledge of the, of the disease causing state or otherwise, they would fall into the benign camp. Only

a small proportion would wind up falling into the pathogenic side.

FURNESS SC: Can we turn over to page 408 and the heading there is "Literature and Database Use". You referred earlier to the use of databases. The first heading is "Population", then "Disease" and "Sequence Databases". Dr Buckley, can I start with you, were any of those databases used in the work you did?

WITNESS BUCKLEY: Yes, we would routinely use many of these databases, so in the population databases the, the largest one, the most current one is not actually on that list, but it is produced by the same group of people who produced the first listed database, the Exome Aggregation Consortium, they have produced a larger database called - which is called gnomAD and that is the - that is a go to database for much of what we do, simply because it has the largest number of people, often normal people involved from the largest number or the most diverse ethnic populations around the planet.

FURNESS SC: And the disease databases, I think ClinVar was used--

WITNESS BUCKLEY: So ClinVar is a database that records pathogenic, likely pathogenic, variant of - it records the attributions of pathogenicity to variation. OMIM is a database of clinical phenotypes and the variation in those phenotypes and how they may present and what laboratory tests may be useful for diagnosing them and where they exist, recommendations for, for the criteria for diagnosis.

FURNESS SC: And in relation to sequence databases?

WITNESS BUCKLEY: Sequence databases we refer routinely to the NCBI genome reference, the human - the normal human reference sequences. Perhaps the term "normal" is not quite correct, but it is the reference sequence, that is the sequence that is produced from people who have been clinically unaffected by, by a significant disease but normal perhaps is not quite the, the right designation of that. Typical perhaps might be better, rather than normal.

FURNESS SC: Can we then turn to table 3 on page 412, and this is the criteria for classifying pathogenic variants.

WITNESS BUCKLEY: Yes.

FURNESS SC: Dr Buckley, or perhaps another, could take us through how table 3 is to be read?

WITNESS KIRK: So if you look at the evidence of pathogenicity down on the left, the different criteria are grouped according to the strength of evidence, and this was a consensus opinion from a large group of people who have been doing this work for some time, with a lot of consultation with the genetics community, and there has been some subsequent work demonstrating that there is some empiric validity to these classifications. So I don't know if you want me to go through the individual, individual criteria, but essentially they're

grouped based on how strongly they influence an assessment as to whether a particular variant is pathogenic or not, and they're either very strong, strong, moderate or supporting in descending order of the extent to which they contribute to that assessment.

FURNESS SC: Can I draw your attention to moderate?

WITNESS KIRK: Yep.

FURNESS SC: And PM1 and PM2?

WITNESS KIRK: Yes.

FURNESS SC: I take it the "P" is pathogenic and the "M" is effectively moderate?

WITNESS KIRK: That's right.

FURNESS SC: The PM2 absence from controls, what does that mean?

WITNESS KIRK: So we talked about the gnomAD database, and that is the primary source these days of controlled data, so they're individuals who are not known to be affected by a Mendelian condition. They come from a variety of different sources but they are certainly no more likely than the population, probably less likely than the general population to have a disease causing variant in any given gene, although that'll vary according to mutation mechanism and so on. And the interpretation of that has to be taken into - has to take into account the frequency of the condition. So that was the basis in fact for one of the filtering steps was to exclude common variation, and this is the reverse of that.

If you see a variant that is absent from controls or is at very low frequency relative to the condition, that is moderate evidence in favour of pathogenicity. It’s not proof because every time we do Whole Genome Sequencing we discover new variants that have never been seen before, so everyone in the room would have variants that are not in the population databases, and that's why it's only a moderate level of evidence rather than strong.

FURNESS SC: Then table 4 is the criteria for classifying benign variants. Do you see that?

WITNESS KIRK: Yep.

FURNESS SC: The standalone is BA1, allele frequency is greater than 5%.

WITNESS KIRK: Yes.

FURNESS SC: Can you explain that?

WITNESS KIRK: So allele frequency, we talked before about the definition of

allele, and generally speaking we have two alleles, so apart from males and the X chromosome, the expectation is that each person will have two copies of each bit of the genome, and, and that means that if something is present in 5% of alleles, that 10% of people will carry that variant, and it's applied to any given population. So sometimes you'll find something that is common in one population but overall not so common, but the assessment of the group that prepared the guidelines is that if you see something that is so common that one in ten people has that variant, then it is vanishingly unlikely to be associated with any diseased state, and that therefore can be excluded from consideration.

FURNESS SC: In relation to strong, BS2 is observed in a healthy adult individual for a recessive homozygous dominant or X linked disorder. What does that mean?

WITNESS KIRK: So that means that if you have a condition where you expect some manifestations in everyone who's got that condition by a certain age, and you observe a variant in someone who does not have any manifestations of that condition, then that is strong evidence against that variant being relevant to disease.

FURNESS SC: Does anyone else want to say anything about those two tables? Dr Colley?

WITNESS COLLEY: No, I mean I could just say a little bit about the difference between the homozygous and heterozygous and X linked. Just to go back to the fact where you talked about chromosomes, and all our DNA that encodes for instructions which are genes are packaged into chromosomes, and we said there were 22 pairs of chromosomes, and that's because we get one from our - from each of our parents, from our mother and our father at conception. Homozygous refers to the phenotype being caused when there is an alteration, a pathogenic alteration or variant in both copies of that chromosome, the one we got from our mother and the one we got from our father. So we assume that in that case, or we usually test, that both parents would be carriers of one copy of the faulty gene, whereas the person who's affected has both copies that are faulty, so they're homozygous affected.

When a person has a condition that's a dominant condition, they actually have the phenotype when only one copy of the gene is faulty, the one from their mum or the one from their dad, or neither parent may have a faulty gene, it might have just become faulty when they were conceived. And an X linked or hemizygous disorder is one in where the faulty gene resides on the X chromosome and you'll remember that we females have two X chromosomes, so in fact we have a backup copy, so we are not as likely to manifest a genetic condition when the fault is in a gene on the X chromosome. But males only have one X chromosome, because their other 23rd chromosome is that Y chromosome that makes them male. So males manifest conditions caused by faults or variants, pathogenic variants in genes on the X chromosome.

WITNESS BUCKLEY: May I also have a supplementary?

FURNESS SC: Yes, certainly.

WITNESS BUCKLEY: I think the part of the sentence that exists in the guidelines that we didn't refer to was that as observed in a healthy adult individual with - for a disorder with full penetrance expected at an early age. I think the full penetrance at an early age is an important consideration, and an example I gave earlier I added at counsel's - had requested a comment that I had included BS2 for a variant in KAT6A which is a gene, mutations of which cause severe intellectual disability, amongst other features, at a very, very, very early age. So the fact that Kathleen - sorry, the observation that an unaffected person in her mid-50s did not have intellectual disability is critical to the interpretation of that, because the disease is expected to be present in a child, to be fully present at an early, at an early age.

FURNESS SC: What does full penetrance mean?

WITNESS BUCKLEY: It means - penetrance is the concept that if you have the variant and that variant is able to cause disease, that it reflects the likelihood of that. So if you've got the variant - if every single person who has that variant gets the disease, then it's 100% penetrant. If only one in two get that disease, then it's 50% penetrant, and that difference may be due to environmental factors. For example, some people have got very high - have got genetic predispositions for getting high cholesterol levels. That's fantastic, but if you can somehow eliminate cholesterol from your diet then you don't get a high cholesterol level. So you can be non-penetrant despite having the variant.

FURNESS SC: Moving on from the standards and guidelines, unless anyone wants to say anything of a general nature about them, because we'll come back to them in respect of the individual variants? Yes Dr Buckley?

WITNESS BUCKLEY: There is one, there is one other comment I'd make. Some of these guidelines - and it's poorly defined, that if you have, for example the - if you go to table 3 on page 412, you go to PS3, it's about a third of the way down the page, PS3 reads, are "well-established in vitro or in vivo functional studies supportive of a damaging change on the effect of the gene or the gene product." The guidelines do not define what a well-established - what are the characteristics of “well-established” in that case. So there is - although the guidelines are powerful, they are also not - what's the word--

WITNESS KIRK: They're subject - they are subject to some interpretation.

WITNESS BUCKLEY: They're not prescriptive in a sense, yes.

FURNESS SC: Having referred to the various evidence in table 3 and table 4, it's the case isn't it that clinical judgment, your clinical judgment, plays a significant role in determining whether or not the evidence of pathogenicity is strong or less strong?

WITNESS BUCKLEY: I think that's true.

WITNESS KIRK: Yeah.

FURNESS SC: They're guidelines, they're not prescriptive mandated matters that one must follow--

WITNESS KIRK: Yes.

FURNESS SC: --without exercising proper clinical judgment, that's right?

WITNESS KIRK: That is certainly true, and there are occasions when it is appropriate to upgrade or downgrade the strength of evidence which you apply, so that you might think - you might find for example - a good example is segregation data, so that's tracking a variant in a family, and that's listed at quite a low level in, in the data. But there are some circumstances where that can actually provide very strong evidence for or against pathogenicity of a variant, depending on family size and exact way that it had, that it goes in the family, and so it may be quite appropriate to upgrade that to moderate or strong evidence, depending on the specific circumstances.

FURNESS SC: Can I turn back to your joint report at page 8. You there set out the analyses which were performed, and the first was hypothesis free whole genome or exome analysis?

WITNESS BUCKLEY: Correct.

FURNESS SC: What does hypothesis free mean in that context?

WITNESS BUCKLEY: That we did not have a group of genes that we were interrogating to see if there were variants in them, having presumed that, that we - what the disease process was. We chose to look at the data itself to see what emerged from the data and whether that was consistent with a clinical phenotype that has been observed in this family.

FURNESS SC: The funnel that you gave evidence about this morning, had at a middle stage 279 variants?

WITNESS BUCKLEY: That's correct.

FURNESS SC: And they're the 279 variants that are referred to in your first paragraph, is that right?

WITNESS BUCKLEY: That is correct, yes.

FURNESS SC: Then ultimately towards the end of that page you come down to nine variants?

WITNESS BUCKLEY: Yes, there are nine variants that were then selected for further consideration.

FURNESS SC: You describe by reference to that funnel how you went from 279 and ended up with nine?

WITNESS BUCKLEY: That's correct.

FURNESS SC: What you haven't described yet is the use of the OMIM database which you've given evidence about and the PubMed searches, and they're set out in the middle of that page. How do they fit in to what you did?

WITNESS KIRK: The OMIM database is a database of both genes and most importantly of human conditions associated with variation in those genes. It was started by a clinician called Victor McKusick in the 1970s and has been maintained since then with the continuous addition of new information as it's been published. It is compendious but not complete, and that's partly because there is new information that gets published all the time and it's very difficult for the people at the NCBI in the United States to keep up with, with the rapid generation of new knowledge.

However, it is a baseline that we always consult because it's an important resource in the field, and so we looked to see if there were published - if there were phenotypes, conditions in OMIM related to the genes in which the variants were identified. Because of that gap between keeping OMIM up to date and current knowledge, we also put together a search of the published literature, so that would not suffer from that, and that was not entirely hypothesis free. It used a number of search terms that we and the others who were involved in the, in the discussion deemed reasonable to apply, relating to different conditions that might result in sudden death in an, in an infant.

And the result of that was that in the results that were generated, for each variant, we had information firstly about whether there was an entry in OMIM relating to that particular gene, and then what that condition was, and it provides you with the information then you can rapidly go to the database to check whether it may be relevant or not. And also a list of all the published papers that match those terms that also contain the name of the gene. So if you look at the structure of the search, it starts with HGVS symbols, so that's the symbol that uniquely identifies a particular gene, and then after that with all those brackets is listed all the different terms that were applied.

And they, as you can see, span cardiac but also more general terms such as, I think sudden death's in there and also some, some terms relating to problems with breathing, autonomic dysregulation and so on. So, they're quite broad and the intention was to deliberately make them broad, within reasonable parameters, so that the chance that we would miss something that had been very recently published would be as low as possible.

WITNESS BUCKLEY: And at the - at the end of doing that procedure, applying that algorithm to the data set of 279 minus 14, because 14 were only present in Kathleen, we were able to exclude 167 variants in genes where there was really no evidence that they were associated with a disease process

of any type at the time of the search.

FURNESS SC: Coming back to the beginning of that paragraph, "Hypothesis-free", you concluded that of the 279, 21 were present in all four siblings--

WITNESS BUCKLEY: That's correct.

FURNESS SC: --84 in three of the four, 73 in two of the four, 87 in one, and 14 were only present in Kathleen. Do you see that?

WITNESS BUCKLEY: Yes.

FURNESS SC: And you've just explained the basis upon which you've excluded the 14.

WITNESS BUCKLEY: Yes.

FURNESS SC: So, ultimately, you've come down to - after the 167 being excluded for the reasons Professor's Kirk's given, you've got three variants excluded as they were "variants in genes associated with X linked disorders whose clinical features are unrelated to the clinical presentation of the four deceased children". Now, is one of those IDS?

WITNESS BUCKLEY: One of those was IDS and I would perhaps ask my colleagues to expand on, on the reason for exclusion at this point, or--

FURNESS SC: Certainly.

WITNESS KIRK: Would you like me to do that?

FURNESS SC: Yes.

WITNESS BUCKLEY: Yes.

WITNESS KIRK: So, we excluded that one because we had strong evidence that the, the child--

FURNESS SC: Perhaps you might explain what it is first?

WITNESS KIRK: Sorry, yes. So, the variant was identified in Patrick. This relates to a condition called Hunter syndrome, which is a condition in which there is abnormal storage of material in a component of the cell called the lysosome and it's one of a group of, of lysosomal storage disorders. And the effect of this progressive accumulation of material is both enlargement of the tissues that are involved, but also damage to the function of some of the organs, particularly the brain.

This is a condition which is not always clinically obvious in the first year of life, although there may be features present as early as birth, but they are generally

not the most distinctive features of the condition. The reason that we felt confident in excluding this from consideration is that a very - two very sensitive biochemical tests had been done which were not consistent with the diagnosis and we were aware of that information. So, we, we, deemed that it did not need further evaluation.

FURNESS SC: Now, those tests, I think, were in tab 70. If we can have that on the screen?

WITNESS KIRK: Sorry, Dr Buckley's also reminded me that, in addition, we had post mortem evidence that it was not consistent with the diagnosis.

FURNESS SC: Let's start with the tests.

WITNESS KIRK: Yep.

FURNESS SC: So, this is the Adelaide Children's Hospital report, is that right?

WITNESS KIRK: Yep.

FURNESS SC: And what does that tell you, Professor Kirk?

WITNESS KIRK: A number of different - so, first of all, I should say something about the laboratory. So, this is one of the leading laboratories for the investigation of metabolic conditions in Australasia and really worldwide for some of these conditions, particularly the lysosomal storage disorders. The investigations which have been done, most of the top part there is not relevant to this, they're there - tests for various other conditions. And, in fact, I think we need to go to the next page.

FURNESS SC: Just before we do that--

WITNESS KIRK: Sorry, yep - go, go on, sorry.

FURNESS SC: --let's go back to the first page.

WITNESS KIRK: Yep.

FURNESS SC: So, the report is dated 8 November 1990?

WITNESS KIRK: Yes.

FURNESS SC: And Patrick was born on 3 June 1990. So, that tells us how old he was at the time of the test?

WITNESS KIRK: Yes.

FURNESS SC: And the test was referred to by a paediatric neurologist?

WITNESS KIRK: Yes.

FURNESS SC: So, you would take it from that that Dr Wilkinson asked for this test to be done on Patrick?

WITNESS KIRK: Yes.

FURNESS SC: So, continue. Page 2?

WITNESS KIRK: So, the - at the top of page 2, there's the urine result from 25 October 1990 and that's the mucopolysaccharide screen. So, the conditions are known as mucopolysaccharidoses and that relates to a chemical - a group of chemicals, four different chemicals, that are found to varying degrees in different members of this group of, of disorders and the, the two tests that were done are a semi-quantitative MPS test. So, that is a test which measures the total amount of glycosaminoglycan and mucopolysaccharide material, and it relates it to the amount creatinine in the urine and that is important, because if you measure something in urine, obviously urine may be more or less concentrated on a particular day. So, the absolute number, if you relied on that, might be misleading and creatinine is something that's a good measure of the concentration of the urine and so the result is normalised against that.

This test is a very sensitive test for all of the mucopolysaccharidoses but doesn't narrow it down to any particular one or two. The laboratory at the Children's Hospital at Westmead instituted the same test in 1997 and we are not aware of any false negative results in that time. The test is set to be sensitive and so it includes other - potentially, can include other compounds that can give you a falsely high reading, but I'm not aware of falsely low readings being recorded, although of course every test has its limitations. Nonetheless, this is the primary screening test that we use in New South Wales for screening for the mucopolysaccharidoses and although we - as I say, we see reasonably frequent false positives, false negatives are so rare that we normally would not investigate further if we received such a result.

In addition, the laboratory had done a, a second test - and I think that was just reflective of their practice at the time rather than any lack of confidence in the first test - which is a qualitative MPS pattern by high resolution electrophoresis. Essentially what they did was that they separated out the compounds of interest, they used an electrical gradient - so, a positive and a negative charge across a membrane - and used that to draw the compounds of interest across the membrane. There was then staining done and you - what you get is a pattern that is very distinctive in people who have MPS conditions, as long as the urine's not too dilute and, as I say, the lab would be very aware of that.

So, that gives you information both about the presence or absence of an MPS disorder, but also about the likelihood of which of the group it is. So, for example, you get very similar patterns in MPS type 1 and type 2, but type 3 has a distinctively different pattern, and so it goes part way to answering the question of which of the conditions you might be dealing with if it's positive.

FURNESS SC: Come back to IDS.

WITNESS KIRK: Yep.

FURNESS SC: How does everything you've said relate to whether or not Patrick--

WITNESS KIRK: It's very strong evidence against him having had any pathogenic variants in IDS.

FURNESS SC: There's also reference that Dr Buckley wishes to make to the post-mortem?

WITNESS BUCKLEY: I was purely reminding Ed that, that a post-mortem had been conducted and that there was no evidence on post-mortem that would be consistent with Hunter syndrome, although that is well out of my area of my competency to speak on. Perhaps, Edwin, would you--

WITNESS KIRK: Yes, I have reviewed the post-mortem report and they were - there was a tissue examination of multiple tissues in which you would expect to see storage. In the first year of life, that may be relatively subtle, it's not impossible that it could be missed by a pathologist. I understand that the slides have been viewed by multiple pathologists and I think it's unlikely that, that they - that between them they would have missed this, but it's not inconceivable. Nonetheless, that's another piece of evidence against that having been the diagnosis.

And then, lastly, I would say that, as far as I can tell, none of the information I received about Patrick in any way connects this condition to the events of his life and death.

FURNESS SC: Dr Colley, did you want to just say anything about that?

WITNESS COLLEY: I would only agree, absolutely, that clinically, from a phenotypic point of view, there was no evidence that Patrick had Hunter syndrome at all and when people do - young boys do die of Hunter syndrome, it's not from having a variation in the gene, it's from actually having the condition because of the mucopolysaccharide that's been stored and affected the tissues, and there is clearly no evidence of that.

FURNESS SC: Thank you.

WITNESS KIRK: Sorry, I should - may I?

FURNESS SC: Certainly.

WITNESS KIRK: I should say that it's a condition that's progressive over years and that death may occur in the first decade of life, although it can be later than that as well. But it's something that occurs after a long period of progression of, of symptoms which are very striking, very prominent. It's not a

condition that you miss.

FURNESS SC: Thank you. Now, we'll come back to the nine variants that have been identified as a result of the hypothesis-free process. Can we turn to the next page, which is page 9? And this, you refer to having conducted a "gene panel analysis" or "analyses". Can you explain that?

WITNESS BUCKLEY: So, these were a group of analyses which were hypothesis driven, that we, we selected genes that were known to be associated - that had been published in association with sudden, sudden death in infancy, and they fell into both cardiac and non-cardiac genes. We separately analysed a list of 204 genes, that was provided to us by a neurologist, for neurological disorders and separate and not mentioned on this particular report because it was done subsequent--

FURNESS SC: Just let me stop you. You need to just get a bit closer to the microphone, if you can.

WITNESS BUCKLEY: Sorry.

FURNESS SC: Let me just go back a step. So, there were two analyses done, one was in relation to 204 genes associated with neurological disorders, that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: And another in relation to cardiac/non-cardiac genes, yes?

WITNESS BUCKLEY: That were - that had been published in association with sudden death in infancy, sudden unexplained death.

FURNESS SC: The first one - that is, first by reference to your report - the 421 cardiac/non-cardiac genes, why did you do that?

WITNESS BUCKLEY: Really, it was a - it was a, a, a check on the hypothesis-free strategy. Because there was evidence that these genes could be associated with sudden unexplained death in infancy, and we were conscious that we in particular use the CAD score to filter out some variants, we repeated the test - the analysis rather, using this candidate list of genes, but we also dropped the use of the CAD score, so that we could examine all the variants that met the frequency criteria in that it's--

FURNESS SC: You gave evidence earlier that the expert panel as a whole - that is, the Canberra and the Sydney teams - used a CAD filter stringency of ten?

WITNESS BUCKLEY: Yes, well, I think Canberra uses one of 12, but it's, it's a, a small numerical difference, but it--

FURNESS SC: What effect does it have to reduce it to zero?

WITNESS BUCKLEY: It admits a larger number of variants into consideration.

FURNESS SC: You identified 32 variants?

WITNESS BUCKLEY: That's correct.

FURNESS SC: And you reviewed each of those variants?

WITNESS BUCKLEY: I did, yes.

FURNESS SC: You reviewed them as against what benchmark?

WITNESS BUCKLEY: So, using essentially the same benchmarks as for the previous hypothesis-free, I confirmed that they were real variants, that they had the characteristics of, of, of being high confidence, and that there was then evidence that they were genuinely associated with, with sudden unexplained death in children.

FURNESS SC: You don't refer to pathogenicity at all. Is that a concept that was not relevant to what you were doing?

WITNESS BUCKLEY: No, sorry, I - we did refer to pathogenicity and that is listed then in the table, appendix 10, is it not?

FURNESS SC: So, the appendix gives us the detail of it--

WITNESS BUCKLEY: Yes.

FURNESS SC: --but in relation to what you've said in the body of your report, the benchmark - if I can use that word again--

WITNESS BUCKLEY: Yeah.

FURNESS SC: --was relevant to the guidelines and the standards of that pathogenicity?

WITNESS BUCKLEY: Yes. Yes, correct.

FURNESS SC: That's right. So, it's the same benchmark as you used in the hypothesis-free?

WITNESS BUCKLEY: Exactly so, yes.

FURNESS SC: Now, the 204 genes associated with childhood neurological disorders were provided by Dr Fahey?

WITNESS BUCKLEY: Yes, Dr Fahey is a clinical neurologist and he wished to ensure that those genes had been examined.

FURNESS SC: Many, if not most of them, had been, hadn't they?

WITNESS BUCKLEY: Yes, I believe so.

FURNESS SC: But, nevertheless, they were done again?

WITNESS BUCKLEY: They were done again, independently, and with a reduced CAD score in this instance.

FURNESS SC: And eight variants were identified.

WITNESS BUCKLEY: That's correct.

FURNESS SC: And, again, the guidelines were--

WITNESS BUCKLEY: Using the same--

FURNESS SC: --applied?

WITNESS BUCKLEY: Using the same application of the guidelines that we used for the hypothesis-free components of the analysis we then re-reviewed those as well, so--

FURNESS SC: Then you did a pathogenic annotation analysis. What's that?

WITNESS BUCKLEY: So, not - there are a very, very small number of, of, of types of - so, mechanisms of pathogenicity which are not due to missense changes or stock changes or splice changes in genes, but are what we call synonymous changes. They change subtly part of the codon, which then leads to a difference in splicing. It's not generally picked up using the, the standard filtering mechanism and I wanted to make sure that we hadn't missed a known pathogenic variant in these genes. In fact in any - for any condition and so we went looking for all known pathogenic variants in these children, just using the abbreviation of the term "path" we just used "path" just to find "pathogenic" or "likely pathogenic". So it's, it's a further check that we hadn't missed anything. So we had hypothesis-free, hypothesis-driven and then a double check to make sure that - or triple check to make sure that, that nothing had escaped our detection, because we had assumed that it was going to be a, a sequence change.

FURNESS SC: Eight variants were identified?

WITNESS BUCKLEY: Yes.

FURNESS SC: And you deal with those later on in the appendix as well.

WITNESS BUCKLEY: Yes.

FURNESS SC: And then finally you did a chromosomal microarray analysis?

WITNESS BUCKLEY: So the chromosomal microarray data was then - was produced by the AGRF and the most experienced units in the city with that particular type of SNP array is actually based at the Children's Hospital Westmead and so what I asked the principal scientist at the Cytogenetics Department at the Children's Hospital Westmead to do was to analyse the data on our behalf.

FURNESS SC: We'll come back to the conclusions you drew and the opinions you formed about that material in a moment, but can I first take you back to the limitations that you've set out on page 7 of the report? Who would like to explain the limitations?

WITNESS BUCKLEY: I think we'll probably all need to contribute, I think. It's - the - I think clearly we, we are analysing this data at a point in time, that we are using the human reference genome that's provided, the hg19. We assume that that is correct, although there may be some minor points where, where it is not correct. We are basing our, our examination on particular DNA samples taken from particular tissues and we assume that a blood - that the DNA that's circulating in our blood is the same constitution as what is in our brains or what is in our hearts or what is in our fingernails, but it's still an assumption. It's likely to be a very, very good assumption, however.

We have also - we are dealing with tissue which has been taken from a child at a point in the distant past, 19 - as late - sorry, as early as 18 - sorry, 1989 and that there will be some deterioration in that, in that DNA, inevitably, as a result of storage and as a result of fixation or as a result of freezing. We also are looking at genes as - we are, we are limited by some structures in the human genome, particularly complex or low complexity repeat regions, which means that it's difficult to map sequences and we are almost always unable to, to identify by Whole Genome Sequencing or Whole Exome Sequencing, large chromosomal rearrangement, so a part of chromosome 1 tacked onto chromosome 2. It's, it's just too large a variation to be seen, though the cytogenic test - previous cytogenic tests have excluded those, in fact.

So I think also it's, it's true to say that not all disease-associated genes have yet to be identified and that the clinical significance of variation in many genes is, is not fully understood. Edwin and Alison, would you like to--

WITNESS KIRK: Yeah, well I agree with all - yeah, I agree with all of that. Only I think I'd summarise it by saying that although these are very, very good tests, they are not perfect.

WITNESS BUCKLEY: Yes.

FURNESS SC: Dr Colley?

WITNESS COLLEY: I would agree.

FURNESS SC: So it's not the case that this exercise has been pointless, because of the extent of the limitations?

WITNESS BUCKLEY: No.

WITNESS COLLEY: No, not at all.

WITNESS BUCKLEY: I would say to the contrary, I think it's been a remarkably informative test and it's yielded surprisingly good results, I think, given the age of the samples.

FURNESS SC: By "good" you mean what?

WITNESS BUCKLEY: That we are able to identify a, a significant number of variants and that the quality of the data looks, looks nice, you - when you're visualise these, in fact you should see some of the illustrations provided, for example, on the top of page 10, in the report the KCNAB2 as an example, you can see that there is good sequence coverage, that the variants stand out, the, the traces are nice and clean, it's, it's - if I had sat here in Court in the year 2000 and said, we will have a technology that can do this on, you know, 30 year old samples in just five years' time, people would have been astounded. This is, this is a remarkable achievement, I think.

FURNESS SC: You referred to deterioration, is there evidence that you have seen that the samples have deteriorated to such an extent that the results are not useful?

WITNESS BUCKLEY: I see no evidence of that.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Not in relation to the Whole Genome Sequencing and exome sequencing. I think one of the arrays--

WITNESS BUCKLEY: Oh yes.

WITNESS KIRK: --the answer is different to that.

FURNESS SC: Dr Colley?

WITNESS COLLEY: I agree with what has been said.

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

WITNESS COLLEY: 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 have 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 am 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.

FURNESS SC: Is the field changing so much that it's pointless to express an opinion today?

WITNESS 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.

FURNESS SC: Dr Buckley?

WITNESS BUCKLEY: Yes, as you stated in your opening address, this is a multifaceted test with clinical components and laboratory components and interpretive components. The clinical component is going to be the same in another five years. The features of these - of this family is of well grown, developmentally normal children who have a sudden and catastrophic event, but without many features of a genetic disorder of early childhood onset. That clinical setting, together with the power of the Whole Genome Sequencing result in combination, I think means it is very unlikely that despite the advances and we will expect that there will be new diseases, but I think that the new diseases that are discovered are not going to be relevant to this clinical situation. So I - anything is possible but in my professional opinion I think that the likelihood in this particular situation is quite low.

FURNESS SC: Thank you. Professor Kirk?

WITNESS KIRK: Yeah, I'd concur with my colleagues. Clearly you can't exclude the possibility that next month there might be a paper that comes out that describes a genetic condition that could describe exactly this situation. If that were the case, you would imagine that it would relate to cardiac disease or to a neurological condition, most likely so I would defer to my cardiac colleague and, and to the neurologists about the likelihood of that. But certainly from what I know of that group or those groups of conditions and from what I've seen during my career, I think I agree, that it's, it's very unlikely that we're going to identify something in the future that will, that will explain this.

FURNESS SC: Professor Skinner, do you have anything to say about that topic?

WITNESS SKINNER: Yes. The principles aren't really going to change, I don't think. If you have four very young children who have a catastrophic event, then the parent, if they carry the same genetic marker, would not be expected

to be alive. And also if - no matter what the disease. If the - neither parent had the genetic marker and all four children did carry the same genetic marker, then maybe that could explain it, because we would have a disease which is so severe, which has killed four children, but is absent in the parents. But the chance of that happening is very small, because these conditions are usually de novo. So these are what, I think Dr Colley referred to earlier, earlier on as, as, you know, very severe, occurring only de novo in that one child at, at conception. So the chance of that happening four times in a row would be remote, because there would be four de novo changes.

The other alternative would be what is called a germline mosaicism, which we haven't talked about, but where there's a change in, in, in the ovum or in the gametes basically, so that neither parent has it in their body, but they, they have it in their - in the, in the sexual reproductive organs, as it were. So it's possible theoretically that you could have four children with a very severe condition that hasn't presented in the adult and that's really the only scenario or the only scenarios that I think that it's likely to work. So in terms of moving forwards, I imagine there will be new diseases and new conditions, but the same principles are going to apply and I think we'll be looking at that when we come to analysing some of these particular variants.

FURNESS SC: What does that give rise to, Dr Buckley?

WITNESS BUCKLEY: So we have done Whole Genome Sequencing in four and exome sequencing in one person. In the event that there was a de novo variant present in all four, we would have seen it.

FURNESS SC: And you didn't?

WITNESS BUCKLEY: I didn't.

FURNESS SC: Professor Kirk?

WITNESS KIRK: I just wanted to add that autosome recessive inheritance is also a possibility where parents are healthy carriers and a child inherits a faulty variant, a pathogenic variant from each parent. Again, we didn't see that but, but that is another mechanism that could be considered.

FURNESS SC: Just before I come to the results, in addition to looking at the cardiac/non-cardiac genes that have been published in relation to sudden death and the neurological disorders, you also carried out an exercise in relation to immunological disorders. Can you tell us about that, Dr Buckley?

WITNESS BUCKLEY: Yes, so we were at - during the analysis period it was raised that, that in particular, variants in some of the interleukins would be - had been canvassed in the literature and counsel wished us to examine the possibility that there was an immunological cause of this phenotype. They would have been captured in the hypothesis-free. We hadn't made that hypothesis, so by definition they should have been in there. However, we obtained a list of - from the International Union of Immunological Societies,

from their 2018 list of immune disorders, we used that particular list of conditions and genes to, to search deliberately for any immunological - sorry, a variant in an immunological gene that could be associated. We found a small number of variants--

FURNESS SC: Just before you get there Dr Buckley, I think the Inquiry provided you with a list of genes to consider as well as those that--

WITNESS BUCKLEY: Yes, that's correct, as well, yes.

FURNESS SC: We might, perhaps not this afternoon, but we might make it clear what those genes were in due course. I interrupted you. So as a result of the work you did?

WITNESS BUCKLEY: We found a, a number of variants, of which the only one that I considered had also been identified in the Canberra report and has a particular clinical phenotype. It is - sorry, I'm just looking for the--

FURNESS SC: Are you talking about NLRP1?

WITNESS BUCKLEY: Yes, I'm just trying to find it in this mass of data I'm afraid.

FURNESS SC: So that's the only variant you found that what?

WITNESS BUCKLEY: That we thought was - that met the criteria for consideration, and I then concluded that it was not responsible for the deaths of these, of these children.

FURNESS SC: We'll come back to that particular variant shortly. The Canberra report authors provided a supplementary report which I think each of you have seen, is that right? I take that for a yes?

WITNESS BUCKLEY: Yes. Excuse me, would it be possible to have a short break for two seconds?

FURNESS SC: Certainly.

JUDICIAL OFFICER: Yes. How long do you need?

WITNESS BUCKLEY: Three minutes.

JUDICIAL OFFICER: Five minutes.

SHORT ADJOURNMENT

FURNESS SC: Thank you, your Honour. The supplementary report by the Canberra team, if we can have that on the screen? I haven't tendered that, I'll do that tomorrow your Honour, but if it can be clearly indicated that it's their supplementary report which was, it's undated, but was recently received.

You'll see from this report that on page 2, which is not numbered, but it's the second page, there's reference to the candidate gene analysis, do you see that? Yes?

WITNESS BUCKLEY: Yes.

WITNESS KIRK: Yep.

WITNESS COLLEY: Yes.

FURNESS SC: Then there's sudden unexplained death by infancy genes, 421 genes, yes?

WITNESS BUCKLEY: Yes.

WITNESS COLLEY: Yes.

FURNESS SC: And neurology genes 506 unique neurology genes?

WITNESS BUCKLEY: Yes.

FURNESS SC: Immunology genes, 426 genes.

WITNESS KIRK: Yes.

FURNESS SC: And metabolic genes is on the next page, 435 genes. You understand from this report that it was a process of putting together Sydney laboratory work, Canberra laboratory work, plus the various other genes that were provided through one form or another to come up with ultimately 1,389 unique candidate genes for analysis?

WITNESS BUCKLEY: That's correct.

FURNESS SC: That's the number more or less that you agree was considered in the exercise?

WITNESS BUCKLEY: Yes. I would also make the further point that the diagrams are quite misleading.

FURNESS SC: Yes?

WITNESS BUCKLEY: If you see the overlap, for example on the page that we're currently on screen, you can see that the number of genes in the overlap is 317 and occupies about 10% of the visual on that graphic, whereas 22 is in the side, the, the representation could, could be, could be more accurately that, that in fact that the two teams share the vast majority of genes in common that we're looking at the same things, and it's only a small number which we have chosen to include uniquely into one list or the other list.

FURNESS SC: So one should have regard to the numbers rather than the

diagram?

WITNESS BUCKLEY: I think so. I think the visual impact makes it look as though we're looking at completely different gene sets whereas in fact that's not correct. We are largely overlapping.

FURNESS SC: Professor Kirk?

WITNESS KIRK: I just wanted to say also about these gene lists, that it shouldn't be implied from a list of 1,389 genes that there are that many genes that are really plausible candidates for this scenario. These lists are very inclusive and there are many, many genes which if you examine them closely you would not include. I'm not saying it's inappropriate to have done this, because there are lists that have been compiled by various sources, and it's an exhaustive and exhausting process to go through one by one and exclude them, whereas it's relatively straightforward to apply them and then look at what the results show you. I just wanted to make that point that, that it may appear looking at those numbers as though there are actually that many, that many plausible candidates which is not the case.

FURNESS SC: I think you arrived at the figure of nine candidates that were worthy of closer attention?

WITNESS KIRK: Yes.

FURNESS SC: That's right?

WITNESS KIRK: Yes.

WITNESS BUCKLEY: Mm-hmm.

FURNESS SC: Can we turn then to appendix 1 of your report which is page 10. This refers to the KCNAB2 variant.

WITNESS BUCKLEY: Yes.

FURNESS SC: Perhaps Dr Buckley could you take us through what we see in appendix 1?

WITNESS BUCKLEY: So as described on the page we identified a variant in the KCNAB2 gene that has - that gene has a number of different messenger RNA transcripts which are expressed in different tissues and of interest is the particular, is the cardiac transcript. The gene involves a potassium channel and it's thus an area of perhaps Professor Skinner's particular interest. It was thought that this gene was a cause of epilepsy, and that it perhaps was the cause of epilepsy in a reasonably common chromosome microarray identifiable syndrome, the 1p36 deletion syndrome. But really when that has been exhaustively examined it was found that it was not consistently involved in that deletion of chromosomal material in children with the clinical features of 1p36 deletion - sorry, in the epilepsy part of that thing, of that syndrome, and

therefore it has been ruled out as a cause of epilepsy for the 1p36 syndrome.

FURNESS SC: Let me just stop you there. If we start at the beginning we see that the variant is present in Caleb, Sarah and Laura Folbigg's samples?

WITNESS BUCKLEY: That's correct, yes.

FURNESS SC: It was not present in Kathleen or Patrick's samples.

WITNESS BUCKLEY: Yes.

FURNESS SC: What does that tell us?

WITNESS BUCKLEY: That tells me that the most likely source for this variant is that it has been inherited from Craig that's been--

FURNESS SC: What else does it tell us about what Patrick or Kathleen may or may not have?

WITNESS BUCKLEY: It means that it's very unlikely that Kathleen and - because they - because all of the children have the same in broad terms presenting features of sudden unexplained death in infancy, that the presence of this variant can't explain whatever happened in Patrick.

FURNESS SC: This I think might be over to you Professor Kirk. In addition to the question which has been ruled out of epilepsy, there was a single study with Brugada syndrome?

WITNESS KIRK: Yeah, and so Brugada syndrome is a disorder of cardiac rhythm and Professor Skinner may want to comment about that condition, which is - it's a condition where a relatively small percentage of cases have a known genetic cause, about a quarter, maybe a little more, and it is a, a disorder of cardiac rhythm that can cause cardiac arrest. So that was the - that was the main reason for considering the variant further.

FURNESS SC: Professor Skinner?

WITNESS KIRK: Sorry. Well I've never seen KCNAB2 and I think as Professor Kirk states, I think there's only one family and - with this variant causing Brugada syndrome. So whether it really does or does not is still open for discussion. I think we need to see more families with this before we can even really be sure it does cause this phenotype. For example, if we were looking at a family where we definitely had Brugada syndrome, which tends to affect, by the way in sudden death terms it tends to affect adult males between about the age of 20 and 40, then we would be looking at sodium channel - a gene called SCN5A which no important variants were found in this study. So I think - theoretically possible, but very unlikely I would have thought as a potential cause of the death of these three children.

FURNESS SC: Well, you gave evidence earlier about the Brugada disease

and whether, in your view, based on the material, you'd seen any of the children had features of that condition?

WITNESS SKINNER: Well, as - if I understand it, it's Caleb, Sarah and Laura who have this variant, so Patrick is the only one with a good ECG. So, I can't exclude it on the basis of that ECG. But I would say that it, like any of the other familial conditions, then one would expect some sort of variable. You wouldn't expect them all to die at this very young age in, in a family cluster like this. You would be expecting to see an uncle who died at the age of 40 and a - maybe a child death in the wider family. I think it would be remarkable and, and undescribed to see three infants dying from Brugada syndrome.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yeah, I should have said that the, the history of Brugada syndrome has been that, apart from that one gene, SCN5A, it's been plagued by reports - single reports - that are then never replicated, and there are a small number of genes where - apart from SCN5A, where there has been replication, but the great majority of proposed associations with this condition have turned out to be spurious. I, I - can I talk about gene phenotype evidence and the ClinGen criteria in relation to this variant?

FURNESS SC: Well, certainly. Well, that comes down to the literature section?

WITNESS KIRK: Yep.

FURNESS SC: Have we finished with the clinical review? Is there anything anyone wants to say about that? No? Yes, the literature, "The variant is absent"?

WITNESS KIRK: Yeah, so - and this is the point we made in relation to a variant in another gene - sorry, this is a broader point than about the specific variant, and we made it in relation to a variant in MYH6 but probably should have made the same point here. There, there had been many, many associations between variants in particular genes and clinical conditions reported that then turned out not to be correct. So, just seeing something as an association on one occasion, or even on a couple of occasions, is not proof that that association is solid and real.

As a result of that, there is a group called - an international group called ClinGen, that have drawn up criteria for assessing the strength of evidence for a relationship between a particular gene and a particular condition. And, and, really, the whole process of variant classification can only begin in the circumstance where you've got a clear association between the gene and the condition, because otherwise it's a meaningless exercise really.

In this case, the evidence that we found for this association would really be classified as limited under the, the ClinGen criteria and we, we probably should not have actually gone to the point of, of classifying this and the - using the,

the - sorry, the ACMG criteria to classify the variant, because of that.

FURNESS SC: Well, if we then turn over the page and there's a summary--

WITNESS KIRK: Yep.

FURNESS SC: --Dr Buckley or Professor Kirk, do you want to talk to the conclusion that you've set out in the summary?

WITNESS BUCKLEY: So, the summary is that the only strong evidence that I could find really to support that this might - within the, the pathogenicity framework, that this might be considered, is that it is either entirely absent or at extremely low frequency in normal population of trials. We, we do - it - although we use the term "absent", we do allow one - in one instance, because that instance that's recorded in the database might actually be a false positive record in the database, so we allow a very low frequency in the normal population databases just as a caution. And, even in that setting, the only evidence within the ACMG pathogenicity framework that we could find that would be consistent was that.

WITNESS KIRK: Which is immoderate.

WITNESS BUCKLEY: And it's immoderate. But as Edwin says, the, the framework should - is, is best applied in the situation where you've got a clear association between a gene and a phenotype. This is not that situation. This is - here you could say that the evidence of an association between the gene and the phenotype is disputed.

FURNESS SC: Professor Kirk, looking at it now, what conclusion would you draw based on the evidence you've just given?

WITNESS KIRK: This variant is very unlikely to be relevant to the deaths of any of the children.

FURNESS SC: And is that different from what's said in the summary?

WITNESS KIRK: It's an interpretation of what's said in the summary, I guess.

FURNESS SC: So, is there anything in the summary that you would wish to change?

WITNESS KIRK: Well, I, I guess I would add that, based on the lack of evidence for a gene - the limited evidence for a gene phenotype relationship and, even if you accepted that relationship, the limited - the very limited evidence for disease causation, that it's, it's most likely that - more likely that this is a benign variant. Formally, you'd call it a variant of uncertain significance but, but my interpretation is that it's much more likely to be benign and unrelated to the deaths of the children in which it was - in whom it was found.

FURNESS SC: Dr Buckley, do you agree with that?

WITNESS BUCKLEY: I'd agree with that.

FURNESS SC: Dr Colley?

WITNESS COLLEY: I agree with that.

FURNESS SC: Can we turn then to appendix 3, skipping over appendix 2 for the moment? This is TTN variant 1?

WITNESS BUCKLEY: Yes.

FURNESS SC: Now, turning to appendix 3, if we can have 3 on the - now, Dr Buckley, this variant was present in all individuals - Kathleen, Caleb, Patrick, Sarah and Laura - that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: What can you tell us about this variant?

WITNESS BUCKLEY: This variant is in a gene which is a - it's the largest gene. It's - it has a very, very large number of amino acids. It has a lot of--

FURNESS SC: Sorry, a large number of--

WITNESS BUCKLEY: Amino acids, the constituent units of a protein.

FURNESS SC: Thank you.

WITNESS BUCKLEY: So, it encodes a very large number of amino acids. Everyone who deals with Whole Genome Sequencing and Whole Exome Sequencing sees in almost every single whole exome or whole genome sequence that they are dealing with, there will be one or more variants in the titin gene. They are something of the bane of our existence because they are, are present in everyone and yet we know, in some circumstances, some types of these variants can cause cardiac disease. It happens that in this instance I don't think there is any evidence for that, it's the wrong sort of variation. It was included because it was a cardiac - there are associations with cardiac disease but, really, I don't see clear evidence that this gene would be - that this variant would cause any cardiac disease.

FURNESS SC: Can I just turn over the page and then we'll come back to the first page? "Population frequency" is the heading and then "Allele count number" and then at the end, "Allele frequency". Now is that a frequency that's greater than the SUDI of one in 3000?

WITNESS BUCKLEY: Yes, it is.

FURNESS SC: And is that consistent with what you've just said about it being

in the population?

WITNESS BUCKLEY: Yes, it is.

FURNESS SC: So, coming back then to the first page, under "Clinical review", you refer to it being "associated with the range of clinical disorders of skeletal and cardiac muscle"?

WITNESS BUCKLEY: Yes.

FURNESS SC: Now, you also gave evidence that it was - did you say it was in the wrong area or there was something about this particular variant that--

WITNESS BUCKLEY: So, the, the only types of variation in the titin gene that are predictive of a clinical phenotype are titin-truncating variants. So, this is not of that type. This is - doesn't - it is not predicted to cause truncation of the titin protein, it is simply a, a spelling mistake within the titin protein and it's the - it's a different sort of mutational mechanism. It should not be - it's not predicted to be an inactivating mutation or a loss of function variant in the titin protein.

FURNESS SC: Did you include it because it was present in all five and it was cardiac related?

WITNESS BUCKLEY: That's exactly why I included it.

FURNESS SC: Dr Colley?

WITNESS COLLEY: Yes, I agree with everything that's said. If we go back to the phenotype of these children - although of course Caleb was only 19 days of age when he passed, but still there was no evidence of him having a myopathy. There was no floppiness generally of his muscles, no muscle weakness. The same with the other three children, prior to Patrick's event, there was no evidence of a myopathy clinically at all. And, of course, we've got the post-mortem which looked at the babies' hearts and there was no either hypertrophic or dilated cardiomyopathy in those reports. So, we don't have a clinical phenotype consistent with a pathogenic variation in, in titin gene.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yeah, and onto that I'd, I'd add that Kathleen Folbigg has had an echocardiogram which showed no features of cardiomyopathy.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: I endorse all of that. Kathleen's ECG and echocardiogram is completely normal and she's 50 years old, she's had plenty of time to manifest a cardiomyopathy and she hasn't.

FURNESS SC: Thank you. And, ultimately, it was classified as "likely benign", that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: Now, turning over to appendix 4, this is another of the titin genes, variant 2, and that was present only in Laura's sample, Dr Buckley?

WITNESS BUCKLEY: That's correct.

FURNESS SC: And what can you tell us about this variant?

WITNESS BUCKLEY: I think this variant is really - is able to be dismissed because of its high allele frequency. So, if you go to below the figure, there is a - sections on the left-hand side entitled "Population" and it says there are 559 variants recorded in the normal population, or this particular variation is seen 559 times in a normal population group. That gives it an overall allele frequency of one in 400, which is not quite but almost ten times more common than, than SUDI. So, it is also independently recorded in various databases as being likely benign or benign, and I would agree with that.

FURNESS SC: Why did you include it?

WITNESS BUCKLEY: Again, because it was in the titin gene and I had thought that it might come up for discussion and I wished to be prepared, I wished to have my - the information marshalled.

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yeah and I agree with all of that.

FURNESS SC: Dr Colley?

WITNESS COLLEY: I agree.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: Yeah, nothing to add there, thank you.

FURNESS SC: Appendix 5 is TAB2 variant and, Dr Buckley, that was present in Kathleen, Caleb and Sarah, but not Patrick or Laura, that's right?

WITNESS BUCKLEY: That's correct.

FURNESS SC: So, what can you tell us about that variant?

WITNESS BUCKLEY: So, loss of function variants in this gene have been associated with congenital heart defects, so structural anomalies of the heart - none of these children do have a structural anomaly of the heart - and they have a range of other features which are listed in the paragraph under the -

under the image. They - there are particular clinical syndromes which are associated with this, a sclerosing skeletal dysplasia, in particular.

This really is ruled out of contention because it is in a relatively small number of people in the normal population database gnomAD, three out of roughly a quarter of a million. But, critically, it's present in Kathleen's sample. Kathleen does not have the features, I am told, of sclerosing skeletal dysplasia, sclerosis of the skull and under model cortices for long bones and phalanges. So, that, I think, makes that a very unlikely variant in this clinical situation. Edwin?

FURNESS SC: Professor Kirk?

WITNESS KIRK: Yeah, vanishingly unlikely.

FURNESS SC: I beg your pardon?

WITNESS KIRK: Vanishingly unlikely to be relevant.

FURNESS SC: "Vanishingly unlikely"?

WITNESS KIRK: Well, I think we can exclude it.

FURNESS SC: Thank you. Dr Colley?

WITNESS COLLEY: I agree.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: It's not my area, I'll defer to the others on this one.

FURNESS SC: Given the view you've expressed, the summary that it's categorised as a "variant of uncertain significance" seems unusual?

WITNESS BUCKLEY: It's the difference between what, what the use of the--

FURNESS SC: Just let me stop you, Dr Buckley, I've been told that in fact this was one of the ones that you revised to "likely benign"--

WITNESS BUCKLEY: Yes, yes.

FURNESS SC: --when the report was corrected.

WITNESS BUCKLEY: Yes.

FURNESS SC: So, by all means, answer my question if you wish to.

WITNESS BUCKLEY: So, the, the strict application of the ACMG guidelines produces results which are - which can be in a - not quite counterintuitive, but where things get classified as variants of uncertain significance because of conflicting evidence or because of lack of evidence. I had not - I - at the start

of this process, I was - we were not given a clear description of whether Kathleen Folbigg had any clinical features and so I erred on the side of caution by not including the, the BS2 criterion, that the presence in an unaffected, normal individual within the family would rule that out. Having been appraised that Kathleen does not have the features that are listed on page 18, I think we're quite capable of using that and so this has now been called "likely benign".

FURNESS SC: Thank you. Yes, Professor Kirk?

WITNESS KIRK: So I just want to say that this does in some way go to inherent conservatism of the ACMG guidelines where there is a bias in the middle end to not overcalling something as benign because of the possibility that new knowledge might come along for any given variant. And in fact if you take any disease gene and any missense variant, so a variant that changes the protein structure, that is absent from population databases. It becomes very hard under the ACMG criteria to classify that as anything but a variant of uncertain significance. With, with the exception perhaps if you've got strong clinical data that help you to interpret that. And so as a result all of us are walking around with many, many variants of uncertain significance which are actually benign.

And so I guess when we think about this, we take a probabilistic approach. Formally we may classify something as a variant of uncertain significance, but in the overall clinical situation we may still form a strong view that it's unlikely to be clinical relevant.

FURNESS SC: Thank you. Turning to appendix 6, the KAT6A variant?

WITNESS BUCKLEY: I would say exactly the same logic applies to the KAT6A variant. This is a, a disorder of childhood onset intellectual disability, it's autosomal dominant. These are very common variants in the, in the population, I think there are 70 or so recorded and it is - I've categorised it as benign.

FURNESS SC: Why did you add it?

WITNESS BUCKLEY: Because again we were - I wanted to make sure that we were looking at every gene which had been described as possibly have a phenotype that was applicable. I wanted to go through the process of making sure that I hadn't missed anything.

FURNESS SC: What was the possible phenotype in KAT6A?

WITNESS BUCKLEY: So they, they have cardiac malformations, atrial septal defects have been reported in, in these.

FURNESS SC: So it was a cardiac related gene?

WITNESS BUCKLEY: It was a cardiac - the, the trigger for, for consideration

was a cardiac trigger.

FURNESS SC: Professor Kirk?

WITNESS KIRK: But in the context of a broader syndrome with other features that were not present.

WITNESS BUCKLEY: Yes.

FURNESS SC: Dr Colley?

WITNESS COLLEY: Yes, I agree. The intellectual disability and the hypotonia is quite marked right from birth.

FURNESS SC: And that was not--

WITNESS COLLEY: Not present in these children. Although, of course, Caleb was only 19 days of age, but again, tone was said to be normal, so were movements and baby reflexes. So we have no evidence of a severe cognitive phenotype in the children.

FURNESS SC: Okay, thank you.

WITNESS BUCKLEY: And Kathleen was--

WITNESS COLLEY: And of course Kathleen and she's alive.

FURNESS SC: That's the BS2, that's right. Turning to appendix 7, this is SCNN1A variant?

WITNESS BUCKLEY: Yes.

FURNESS SC: And it was present in Kathleen and Patrick Folbigg's samples, but not present in Caleb, Sarah or Laura.

WITNESS BUCKLEY: That is correct.

FURNESS SC: What can you tell us about that variant?

WITNESS BUCKLEY: So this is an ion channel and it's possible because of the - ion channels are known to be involved in cardiac disease and are known to be involved in neurological disease and so again it's, it's really an abundance of caution of making sure that we'd considered it, that it was included here. It's present in Kathleen, it causes a disorder which is Liddle syndrome, which I understand - it's actually probably easier if I deferred to either of my clinical colleagues to, to comment on Liddle syndrome.

WITNESS KIRK: Yes, so it's - so, so it's a condition that, as it says in the text, mimics primary hyperaldosteronism, so that's a hormonal condition in which there is an abnormality of a hormone that controls the balance of salts in the

blood and it causes very distinctive abnormalities that would not be missed.

FURNESS SC: Would not be missed?

WITNESS KIRK: Would not be missed in that it affects an individual.

FURNESS SC: And had not been?

WITNESS KIRK: Had not been identified in Kathleen Folbigg, yeah.

FURNESS SC: Dr Colley?

WITNESS COLLEY: Yes, I agree.

FURNESS SC: It also hasn't been reported in the medical literature, is that right?

WITNESS KIRK: This variant hasn't.

FURNESS SC: What is the relevance of that?

WITNESS KIRK: So that's one of the types of evidence that we look at is previous reports of a particular variant in association with a, with a relevant condition.

FURNESS SC: Why then does it become a variant of uncertain significance?

WITNESS BUCKLEY: Because there's not enough evidence to say that it - that this does not cause - when you follow the ACMG guidelines, it gets categorised as, as a variant of uncertain significance, because it's absent from controls, because the computational programs that we use to, to guide us to knowing whether something has a, has an effect on protein structure or function, suggests that it might do, but they are counteractive to - a fair degree, by the observation that it is present in a healthy adult individual.

FURNESS SC: That's Kathleen?

WITNESS BUCKLEY: That's Kathleen.

WITNESS KIRK: To a large degree.

WITNESS BUCKLEY: To a large degree. I would - to answer your question formally, the reason it's a variant of uncertain significance is because it's conflicting information. It's absent from controls. There are indications from pathogenicity prediction software that it could cause disease but we know that it's present in someone who doesn't have that disease. So those data are in conflict, therefore it's categorised as a variant of uncertain significance.

FURNESS SC: Thank you. Can we skip over MYH6, which is appendix 8 and go to appendix 9. This the JUP variant and present only in Sarah's sample.

Dr Buckley?

WITNESS BUCKLEY: So JUP is a known cause of cardiac disease. It causes a number of other disorders as well. This variant has been - it's not been reported in the medical literature, but it exists in ClinVar entries which it is caused - called as uncertain. The only - it's present at low frequency in the European population. There are 17 alleles. It has a frequency of therefore about 1:7,500 overall amongst non, non-Finnish Europeans, Europeans who are not from Finland, sorry. Finland has a particular demographic history. So that is not inconsistent with a cause of sudden unexplained death in infancy. But there's really nothing powerful indicating that apart from the fact that the computational programs predict that it could be, there's really no evidence that it does cause disease.

FURNESS SC: Professor Kirk?

WITNESS KIRK: I add to that that if this were a cause of sudden death, it would have to be a very important cause of sudden death, although the frequency is just about at the level where you could consider that. It, it would be well known as a major cause of sudden infant death and on top of that, the particular cardiac condition is a condition called arrhythmogenic right ventricular dysplasia and the post mortem examination on Sarah did not show any evidence of this condition, which you would expect to be present if it were the cause of her death.

FURNESS SC: Thank you. Professor Skinner, do you have anything to add?

WITNESS SKINNER: Yes, again, if it were arrhythmogenic right ventricular cardiomyopathy, this is not a disorder which causes death in infancy. It causes death from young athletes and typically during exercise and in older age. The Naxos disease can be quite severe, but again, it doesn't cause death in infancy and it certainly doesn't cause sudden unexplained autopsy negative death in infancy.

FURNESS SC: Thank you. Dr Colley, did you want to add?

WITNESS COLLEY: No, nothing more to add.

FURNESS SC: Then can we turn to appendix 10 and this is of a different nature. This a list of the genes or variants identified in the 421 genes associated with sudden cardiac or non-cardiac, as you gave evidence of earlier. Dr Buckley, is that right?

WITNESS BUCKLEY: Yes.

FURNESS SC: So what does this appendix tell us?

WITNESS BUCKLEY: In summary, it tells us that when we take these candidate genes, there is really - there's no compelling data here that says - that stands out saying that this is a clear explanation for what is happening in

these - in this family.

FURNESS SC: So under the heading "Observations", they're the reasons you have for opining as you just have?

WITNESS BUCKLEY: Yes, so they are, they are various, they depend on the, on the particular variant that, that's under discussion. In some instances these have actually been previously observed and have been commented on in databases saying that they are likely to be benign. In many instances, they are simply too frequent if you - if I could just take you, for example, to the second one on the list, the ANK3, blah-blah-blah. It says under "Observations", likely benign and it has an autosomal recessive pattern of inheritance with four normal homozygotes observed in gnomAD.

That observation would rule it out as a pathogenic cause, I think. So the combination of frequency, zygosity, presence in coding or non-coding regions of genes, predictions already commented in the literature or in databases to be likely benign or simply that it causes an unrelated disorder as, as the predominant sort of - so, so you could get, for example, there is a record here of a, of a - of an aortopathy, I think, so a disease of the major vessel that comes out of the heart.

Yes, of course you can get sudden death due to, due to aortic dissection, that would be a common presenting issue here at Coroners Court, I think. But that's not what happened in this family, because we know they've had - they've already had post mortems and we know that that does not apply in this particular group of deaths. So that gene was actually the MYLK variant, sorry, it's an unrelated disorder, a cause of aortic aneurysm.

FURNESS SC: And there's reference to CALM2 which we'll come to and JUP.

WITNESS BUCKLEY: Yes.

FURNESS SC: And MYH6, which we'll come to, and the two titins?

WITNESS BUCKLEY: Yes.

FURNESS SC: So they had been picked up already.

WITNESS BUCKLEY: They had been, yes.

FURNESS SC: Professor Kirk, did you want to add anything?

WITNESS KIRK: No.

FURNESS SC: Dr Colley?

WITNESS COLLEY: No.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: Yes, I think one of the most useful things about this appendix really is the genes that are absent from this. So when I came into this right at the beginning, there were three or four genes that really I felt we needed to really take out of this. Genes that have been associated with sudden infant death. For example, SCN5A, sodium channel disease, this is not found here. Triadin, autosomal recessive, this causes severe disease, could potentially cause cardiac death in infancy. CACNA1C, that's not here and caveolin is another one. So the four top genes that I came into, in terms of causing infant - sudden infant cardiac death and no, no significant variants, no variants have been produced in this list and I find that an important thing to document at this stage.

FURNESS SC: What does that tell you?

WITNESS SKINNER: Well, it tells us that the, the commonest and most plausible genes which cause sudden death in infancy are not present in this family.

FURNESS SC: Thank you. Does anyone want to add to that? No.

JUDICIAL OFFICER: Does everyone agree with it?

WITNESS BUCKLEY: Yes.

WITNESS COLLEY: Yes.

FURNESS SC: Thank you. There's reference at the top two, including 170 from Professor Vineusa, who is the Canberra team, so they were genes or variants that she - genes that she specifically gave you?

WITNESS BUCKLEY: Yes, so she did an independent literature search looking for causes - genes associated with sudden unexplained death in infancy, generated a list of 170 and she very kindly shared those with us, so that we could make sure that we were all doing as much as possible the same analyses.

FURNESS SC: The IDS didn't come up, the IDS didn't come up in this search?

WITNESS BUCKLEY: No.

FURNESS SC: The next appendix is appendix 11 and this is the 204 genes associated with neurological disorders provided by Dr Fahey?

WITNESS BUCKLEY: That's correct.

FURNESS SC: What can you tell us about that Dr Buckley?

WITNESS BUCKLEY: We went through the same process. There are fewer

genes to be looked at. There are 204 compared with 421 so there are proportionately fewer variants identified, and really it's the same story, that they, that again there is no compelling evidence of an association - of a variant present in here that would, that could explain the clinical phenotype that was observed in these children. So - and it's for the same reasons, largely that most of these variants are relatively common in the population, or they involve transcripts that don't actually produce proteins, or they're located in regions of the, of the, they're very deep within introns of genes, or they are already known to be likely benign variants from other data. It's the same set of reasons in reality.

FURNESS SC: Professor Kirk?

WITNESS KIRK: I agree with that.

FURNESS SC: Dr Colley?

WITNESS COLLEY: I agree.

FURNESS SC: Professor Skinner?

WITNESS SKINNER: I'm happy, thank you.

FURNESS SC: Turning to appendix 12, these are the genes that you described earlier today as being sourced using the term pathogenic and nothing else.

WITNESS BUCKLEY: Yes. So this was looking for any variant in this family which had been, had been - that exists in the databases as a cause of any form of disease of any type. Sorry, of any form of Mendelian disease.

FURNESS SC: I'm sorry, can you say that again?

WITNESS BUCKLEY: Of any form of Mendelian disorder, of any type. And if you look for example at the third one on the list, so they are grouped - there are two lines per variant. If you look at the third one down, the one that starts ABCC6, you can see there is a - the description of that variant is p.Arg1418 and then followed by an equals sign. That means that that - and that is the first instance that we've been talking about today of a different mutational mechanism where the, where there isn't a spelling mistake in the protein which is coded, that in fact the protein looks the same but the gene doesn't splice correctly, so it's a different mutational mechanism.

That was particularly the reason why I wished to run this yet further redundant search, to try and find whether we had missed gene - disorders that are due to that mechanism, the pathogenicity, and indeed we found one instance of it. It was a heterozygous variant for an unrelated disorder which is known to be autosomal resistant so a child had inherited - so Kathleen, Caleb, Patrick and Laura had inherited one copy. In order to get the disease you'd actually need to have two.

FURNESS SC: Professor Kirk?

WITNESS KIRK: And in none of these cases would the particular condition be relevant to the deaths of the children.

FURNESS SC: Because of the phenotype of the children?

WITNESS KIRK: That's right, because of the nature of the conditions involved, yeah.

FURNESS SC: Dr Colley?

WITNESS COLLEY: Yes I agree, and I think what Professor Kirk was saying, that even if the children or adults had these conditions, they are not associated with sudden death.

FURNESS SC: Thank you. Professor Skinner?

WITNESS SKINNER: I concur completely, than you.

FURNESS SC: Can I come back to the chromosome microarray testing that was done, and perhaps if we could have tab 67 on the screen. Dr Buckley, you organised for this to happen?

WITNESS BUCKLEY: Yes, I requested - so I've - I was mindful of how I framed the request, so I, I didn't identify the family in the question, so I used the, the coded name there itself. If we can go to the top of that page.

FURNESS SC: Perhaps I'll take you to the letter which is tab 66, that you wrote requesting the information.

WITNESS BUCKLEY: Okay, yep. So I wanted the examining laboratory to have, to be aware of the clinical situation, that this was a nuclear family where there was sudden unexplained death, but I didn't wish to identify the family particularly, nor did I wish to, to raise any particular hypothesis. We had had some discussions about the CALM2 and CALM 6 variants. We had found heterozygous variants - sorry, CALM2 and MYH6 - we had found some variants in those genes and so I wished the laboratory at Westmead to take a look at those to see if they could see any other sorts of mutational mechanism in that, in those, in or around those genes, but I didn't flag, but I said that it should be of non-exclusive interest. By all means look at those genes, but also look as carefully at everything else.

FURNESS SC: Then the results were tab 67, and those were the results for who?

WITNESS BUCKLEY: Yes, so - this is Sarah is it not?

FURNESS SC: This is Sarah, and what were the results?

WITNESS BUCKLEY: No medically significant copy number variant was detected of any type. So not just looking at the CALM2, MYH6 region, but looking right across the genome, they did not find anything which was an explanation for sudden unexplained death.

FURNESS SC: Just remind his Honour, you gave evidence earlier about why this form of testing was useful to do in addition to the whole genome and whole exome.

WITNESS BUCKLEY: It interrogates a different scale of mutational mechanism or - everything that we have been looking at to this point in the Whole Genome Sequencing and Whole Exome Sequencing, you can see a single nucleotide which has changed. In this sort of analysis we're looking for large scale rearrangements of chromosomal material, so duplications not just of a single base but tens of thousands of bases, mega bases in fact on occasion. This test is a well described, well established work horse diagnostic test, of which thousands are done every year in New South Wales. It's a highly robust test and it produces reliable results, and in this case it did not identify any medically significant copy number variant.

FURNESS SC: If we can turn to the next tab, and I think that relates to Kathleen.

WITNESS BUCKLEY: Yes, this is Kathleen's sample.

FURNESS SC: What's the conclusion that was reached?

WITNESS BUCKLEY: It's the same conclusion, that no medically significant copy number variant was detected in Kathleen's sample.

FURNESS SC: The next tab is I think in relation to Patrick.

WITNESS BUCKLEY: Yes, there is a rider here that the quality metrics for these data were somewhat reduced. I guess that's because of the age of the sample or the preservation of the sample, that a single nuclear type is reliably sequenced, but these larger scale variations are not as reliably identified, and so for this particular sample, although they have concluded that no medically significant copy number variant was detected, they have placed some boundaries around that. Edwin, would you like to comment on that?

WITNESS KIRK: Yeah, so the specific boundaries are that they - the evidence they have is that they would be able to detect a variation that involved up to - more than 200,000 bases, whereas for the other two it was more than 50,000 bases.

FURNESS SC: So it is the case then that the results are not as reliable as the others, is that your evidence?

WITNESS KIRK: It's not - well, it's that the size of variation that they can

detect is different, so if there were a variant that were between 50,000 and 200,000 bases in size, it would be detectable on the standard array but not on this somewhat lower quality result. So it has the potential to miss a particular class of variation.

FURNESS SC: Just before we come back to CALM2 and MYH6, Professor Skinner can I take you back to your report. You on page 2 answered a question, "What cardiac genetic conditions cause sudden death where the autopsy is uninformative?".

WITNESS SKINNER: Yes.

FURNESS SC: Perhaps you could take us through your answer?

WITNESS SKINNER: Okay. So most sudden cardiac deaths in children occur in children with structurally normal hearts, which after death looks normal both to the naked eye and under the microscope. These genetic defects are collectively known as the cardiac ion channelopathies. They are disorders in the cardiac cells at the sub-microscopic level. There is rapid movement of sodium and potassium and calcium ions across the cardiac cell wall which are required for depolarisation and repolarisation of the cardiac cell with every heartbeat.

If the channels through which the ions travel are defective, then repolarisation or depolarisation is abnormal and there is a risk of serious ventricular arrhythmia, a rhythm so fast and uncoordinated that there is no output from the heart and sudden syncope, cardiac arrest or sudden death can occur. And the most common types of these are known as long QT syndrome, CPVT, and Brugada syndrome, and there's a long list of genes which can cause these conditions. So the mode of death and the rhythm recorded during transient loss of consciousness due to these conditions is ventricular tachycardia, it means the bottom part of the heart is beating extremely quickly, and/or ventricular fibrillation which is basically a seizure of the bottom part of the heart, and it's not asystole or a slowing of the heart typically, it's the very rapid rhythm that causes the death.

FURNESS SC: You made some general comments about multiple death scenarios, and this is on page 3 of your report.

WITNESS SKINNER: Yes. So I've been doing this sort of investigation for more than 15 years. I head up a national organisation and we've since 2008 been working with the National Forensic Pathology Service, National Coronial Service, investigating all sudden deaths. It's mandated since 2008 that they should be investigated in this way, and I've presided over that for all of this time, and I've never encountered four sudden deaths of children of any age within one family unit.

When there's been more than two infant deaths, there's been circumstantial evidence to suggest suboptimal sleep environment for the infants in such cases. The classic scenario would be the infant wedged between mattresses

or drugs and alcohol in the house, smoking and suboptimal sleep position and things like this. However, there is a small number of cardiac inherited conditions where such a scenario of multiple sudden deaths in infancy is theoretical possible. So I mentioned there that I entered this investigation with an open mind.

We have like others seen families with multiple deaths within the wider family, though to date none have arisen where such a young age has been a consistent feature. Rather, the age and circumstances of death have been more varied.

FURNESS SC: You have some comments and observations about the type of inheritance and I think Professor that you have covered most of those--

WITNESS SKINNER: Yes.

FURNESS SC: --in your evidence today. Is there anything there that you wished to particularly draw attention to that you haven't already?

WITNESS SKINNER: No I don't think so. I think that summarises what I tried to say earlier, yes. So the three types, de novo, consequence of germline mosaicism and combined gene coming down from both sides would be the type of inheritance we'd expect to cause such severe disease in infants.

FURNESS SC: You say over at page 4, you say in passing, this is the first full paragraph, recessive conditions are more common upon metabolic and neurological disease.

WITNESS SKINNER: Yes.

FURNESS SC: Is that in relation to something in particular with Patrick?

WITNESS SKINNER: No, I, I mentioned that because actually it's only recently that we've started to recognise some cardiac conditions which fall into that bracket, and I mentioned earlier there's a condition called triadin knockout syndrome which you get a recessive gene from both sides of the family and if they're both children - I mean the child has both the alleles from either side of the family, then they can get a very severe disease, whereas the parents have no disease. And the - whereas metabolic and neurological degenerative disease is - this is the commonest means that that could occur and I'm sure that Dr Kirk could speak to that better than I could.

FURNESS SC: Thank you Professor Skinner. I note the time your Honour.

JUDICIAL OFFICER: Yes, we'll adjourn until 10 o'clock tomorrow. Mr Morris it's a highly technical area and as I've said it's not an adversarial process that we're going through and I think counsel assisting has said that if you want to talk to any of the witnesses, if that would be of any assistance to you, then you should organise it with Counsel Assisting I think.

LTS:DAT

MORRIS SC: Thank you, your Honour.

JUDICIAL OFFICER: We'll adjourn until tomorrow morning.

<THE WITNESSES WITHDREW

AUDIO VISUAL LINK CONCLUDED AT 4.01PM

ADJOURNED PART HEARD TO TUESDAY 16 APRIL 2019