EXHIBIT AF

Initial Report into the Genetic Sequencing of the Folbigg Family

Inquiry into the convictions of Kathleen Megan Folbigg

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Date: 29 March 2019

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29 March 2019

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Inquiry into the convictions of Kathleen Megan Folbigg
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Dear Commissioner Blanch AM QC

Re: Genetic Sequencing Analysis of the Folbigg Children and Kathleen Folbigg

1. BACKGROUND

1.1 This report is jointly authored by Professor Carola Vinuesa and Professor Matthew Cook.

1.2 Professor Carola Vinuesa became involved in this matter when she was contacted by the solicitors acting for Ms Kathleen Folbigg, who asked if she could conduct genetic sequencing of Ms Folbigg.

1.3 Stuart Gray, of Cardillo Gray Partners, arranged for Professor Vinuesa's team to meet Ms Folbigg. With Ms Folbigg's consent, Professor Vinuesa's team took her personal history, family history, and biological samples (buccal swab and blood sample) for the purposes of genetic testing.

1.4 From the biological samples Professor Vinuesa's team extracted DNA and conducted whole exome sequencing (**WES**) of Ms Folbigg.

1.5 On 2 December 2018 Professor Vinuesa produced a preliminary report in relation to the WES findings for Ms Folbigg. As a consequence of providing that report to Mr Gray, Professor Vinuesa was asked by Counsel Assisting, Ms Gail Furness SC, to join a team of experts who would be responsible for analysing the genetic sequence data of the Folbigg children.

1.6 Shortly thereafter Professor Matthew Cook was independently approached by Counsel Assisting to provide an expert opinion, initially on the progress in genetics since Ms Folbigg's conviction, and then to provide genomics analysis.

1.7 Subsequently we decided that since we are colleagues, and use the same bioinformatics pipeline, we should prepare a combined report.

1.8 In late December and early January, whole genome sequencing (**WGS**) and/or WES of the Folbigg children and Ms Folbigg was conducted by the Australian Genome Research Facility (**AGRF**) and the Victorian Clinical Genetics Services (**VCGS**).

1.9 Whole exome sequencing differs from whole genome sequencing in that the former examines the coding regions of the genome while the latter examines both coding and non-coding regions. Only about 1% of the human genome directly codes for proteins. The other 99% is typically regarded as structural DNA that is primarily involved in regulating the expression of the coding regions.

1.10 The samples used for sequencing by the AGRF and/or VCGS, as well as the type of sequencing conducted, is outlined in Table 1 below.

Table 1 *Type of DNA sequencing conducted on samples of the Folbigg family*

Individual sequenced	Sample used for sequencing	Type of sequencing conducted
Kathleen Folbigg	Blood sample	**AGRF:** WGS
Craig Folbigg	Declined to provide a sample	**N/A**
Caleb Folbigg	Guthrie card	**VCGS:** WGS
Patrick Folbigg	Frozen liver tissue	**AGRF:** WGS
Sarah Folbigg	Fibroblast DNA	**AGRF:** WGS
Laura Folbigg	Guthrie card	**VCGS:** WES

1.11 On 26 February 2019 we were provided with the raw sequence data for each of the individuals listed above.

1.12 Over the course of the past two months, we have analysed the sequence data of the Folbigg family. This has involved a degree of collaboration with the other experts in determining and refining the scope of the analysis, but we have conducted our own independent analysis and used our own bioinformatics pipeline. The opinions

expressed in this report based upon the findings outlined are each our separate opnions and we both agree with the findings and opinions expressed in this report.

2. QUALIFICATIONS

Profesor Carola Vinuesa

2.1 Professor Vinuesa is an Australian NHMRC Principal Research Fellow, Professor of Immunology at the John Curtin School of Medical Research at the Australian National University (**ANU**) and joint Director of the Centre for Personalised Immunology, NHMRC Centre of Research Excellence. She has been an elected Fellow of the Australian Academy of Science since 2015.

2.2 Professor Vinuesa is the Chief Scientist of Canberra Clinical Genomics. Canberra Clinical Genomics is NATA accredited to conduct, among other things, bioinformatics analysis of DNA and RNA sequences, such as those produced by WES or WGS.

2.3 Her primary areas of expertise include immunology, immunogenetics, medical genetics (excluding cancer genetics), autoimmunity, biochemistry and molecular cell biology.

2.4 Professor Vinuesa obtained a medical degree from the University Autonoma, Madrid, in 1993 and undertook specialist clinical training in the UK from 1993 to 1997. This included junior and senior house officer positions in Cardiology, Gastrotenterology, Pediatrics, Obstetrics and Gynaecology, and HIV medicine. She completed a PhD at the University of Birmingham in 2000, and in 2001 she received a Wellcome Trust International Travelling Fellowship to undertake postdoctoral research at the John Curtin School of Medical Research (**JCSMR**) at the ANU. She subsequently stayed on at the JCSMR after completing her initial postdoctoral research, and became a group leader in 2006 and a professor in 2010. Professor Vinuesa's group currently consists of approximately 20 researchers, staff and students, and is funded by a number of national (NHMRC) and international grants.

2.5 Professor Vinuesa has published over 100 articles, being first author or senior author in over 70% (h-index = 46), and has been cited over 10,000 times, with a citation average of 100 citations per publication. Her publications have chiefly been in high impact journals such as *Nature, Cell, Nature Medicine, Immunity, Nature Immunology, Nature Communications* and *The Journal of Experimental Medicine*.

Many of these articles dealt with the genetic causes of disease, albeit predominantly in the context of immune diseases.

2.6 Professor Vinuesa has also received a number of awards for my research, including the Science Minister Prize (2008), the Biogen-Idec Prize (2008), the Gottschalk Medal of the Australian Academy of Science (2009), the Inaugural Elizabeth Blackburn NHMRC Fellowship (2012), the inaugural CSL-Young Florey medal (2015), and the Ramaciotti Medal for Excellence in Biomedical Research (2015).

Profesor Matthew Cook

2.7 Professor Matthew Cook is Professor of Medicine at the Australian National University, Medical Director of Canberra Clinical Genomics, Co-director of the Centre for Personalised Immunology, and practicing clinical immunologist at Canberra Hospital.

2.8 Professor Cook has published more than 90 peer reviewed articles, attracting more than 8000 citations, with particular focus on genetic and cellular analysis of the pathophysiology of disease. Starting from genomics analysis, his team have described three new syndromes, and contributed to characterisation of additional diseases and syndromes.

2.9 Professor Cook has been invited to speak on diagnostic and research applications of human genomics in Australia and internationally.

2.10 Professor Cook is a chief investigator of the Australian Genome Health Alliance, leads a rare disease flagship of this program. He is a member of the National Project Reference Group on Human Genomics

2.11 Over the last decade Professor Vinuesa and Professor Cook have worked together in establishing large cohorts of immune disease patients and carrying out proof-of-principle genetic sequencing experiments on these cohorts to establish the scientific and technological platform that today forms the basis of the Centre for Personalised Immunology and Canberra Clinical Genomics. As a consequence, they have both overseen the genetic sequencing of approximately 2000 individuals and the genetic analysis of hundreds of patients with rare, often undiagnosed, diseases. This has led to genomic diagnoses of numerous patients.

Contributions from Professor Vinuesa's Team

2.12 From Professor Vinuesa's team, Dr Todor Arsov and Dr Marcin Adamski contributed to the analysis of the DNA sequences of the Folbigg family. Their details are as follows:

Dr Todor Arsov
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Qualifications: MD, MS (Mol Med), MS (Gen Couns), PhD

Expertise: Gene disease association identification, gene mapping, monogenic disease diagnosis with particular expertise in epilepsy, variant interpretation

Dr Marcin Adamski
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Qualifications: Ph.D. in Mathematical Modelling in Hydro-Chemistry; M.S.E. (Master of Science in Engineering) in Computer Engineering and Programming

Relevant experience: genome and transcriptome assembly, annotation and analysis; genome variant discovery and analysis; bioinformatics software design; computer programming

3. DECLARATION

3.1 Statement by Professor Vinuesa: I was provided with a copy of the Expert Witness Code of Conduct.[1] I have read the code and agree to be bound by it. I further acknowledge that I have a duty to assist the Inquiry on matters relevant to my area of expertise, and that I am not an advocate for any party, including Ms Folbigg.

[1] Available at: https://www.legislation.nsw.gov.au/~/pdf/view/regulation/2005/418/sch7

3.2 Statement by Professor Cook: I was provided with a copy of the Expert Witness Code of Conduct.[2] I have read the code and agree to be bound by it. I further acknowledge that I have a duty to assist the Inquiry on matters relevant to my area of expertise, and that I am not an advocate for any party, including Ms Folbigg.

4. PREAMBLE

4.1 In addition to being provided with the raw WGS data of Kathleen, Caleb, Patrick and Sarah, and the raw WES data of Laura, we were also provided with, and reviewed, the material sent to us as part of the "Genetic bundle" plus the available (albeit incomplete) cardiology asessements of Kathleen Folbigg and her offspring.

4.2 Several factors about analysing the sequence data for this kindred differ from a conventional diagnostic WES or WGS task. These are as follows:

4.2.1 There is uncertainty regarding the phenotypes that we are seeking to explain with genetic variants. Because of the circumstances of the analysis, and the short turnaround time in which to produce a report, we have not had additional opportunities to characterize and seek to resolve ambiguities about the phenotype by performing more in depth investigations.

4.2.2 The kindred comprises four deceased siblings. Based on the pathology reports provided, there is evidence that not all siblings had similar pathology.

4.2.3 Sudden infant death syndrome is under consideration as a cause of death for one or more of the siblings. The cause of SIDS remains unresolved. It is possible that SIDS is not a single pathophysiological entity. In other words, a variety of pathological processes may result in what is referred to as SIDS. Causal factors might include genetic variants resulting in abnormalities in many different types of proteins.

4.2.4 The genetic hypothesis for SIDS has been explored in several studies. There is evidence for several genetic causal pathways. Genes implicated predominantly affect cardiac function and neurological function, and to a lesser extent, immunity.

[2] Available at: https://www.legislation.nsw.gov.au/~/pdf/view/regulation/2005/418/sch7

4.2.5 Non-genetic factors are often triggers for disease in the context of genetic susceptibility and this can explain different incidence or presentation of disease in individuals carrying the same genetic variants. For example, amongst identical twins with the same genetic make up, often only one of the siblings develops disease, or the phenotypic presentation is different between the siblings, or disease presents at different ages. Amongst all environmental factors, infections are well-accepted disease triggers for many conditions including immune, cardiac, and neurological conditions. In the case of cardiac conduction defects there is good evidence that virus infections can trigger cardiac arrhythmias through various mechanisms.

4.2.6 The autopsy report for Sibling 4 (Laura) has been reported to show evidence of myocarditis. This does not necessarily indicate a monogenic cause of death. Myocarditis may arise as a result of infection or inflammation. There are genetic causes of increased susceptibility to infection (immune deficiency diseases), and aberrant inflammatory responses (autoinflammatory diseases).

4.2.7 The autopsy report for sibling 2 (Patrick) raised the possibility of a seizure (epilepsy)/encephalopathy/metabolic disorder, for which there are many known monogenic causes. Nevertheless, early onset epilepsy is not always genetically determined.

A. Classifying gene variants for clinical action

4.3 A conventional diagnostic genome report describes “pathogenic” or “likely pathogenic” genetic variants. These genetic diagnoses are usually arrived at according to the American College of Clinical Genomics (**ACMG**) criteria (Richards *et al.*, 2015). Pathogenic, or likely pathogenic, variants arrived at using ACMG criteria are typically regarded as clinically-actionable variants.

4.4 A clinically-actionable variant is one for which there is sufficient confidence in causation on which to base clinical decisions or take therapeutic actions, such as, for example, performing a bilateral mastectomy and salpingo-oophorectomy in the case of BRCA1/2 variants.

4.5 Due to the medical and legal implications posed by unnecessary or even harmful treatment, variants are conservatively classified using the ACMG criteria. For instance, a variant is classified as “pathogenic” when there is a >99% probability that

the variant causes disease and as "likely pathogenic" when there is a >90% probability that the variant causes disease (Richards et al., 2015; Plon et al., 2008).

4.6 In the case of the Folbigg children, we have erred on the side of potentially including false positive variants rather than potentially excluding false negative variants. In this instance we believe that lowering the 90% likelihood threshold for causing disease (i.e. the "likely pathogenic" classification) to include VUS with reasonable evidence of pathogenicity is appropriate, given no clinical or therapeutic outcome will follow from the genetic diagnoses (see paragraphs 4.10 to 4.11 below).

4.7 The pathogenicity scoring system set out by the ACMG standards involves examining a range of criteria for each variant and tallying up evidence in support of pathogenicity to determine if the variant exceeds the required thresholds for classification as "pathogenic" or "likely pathogenic". The effect of this scoring process is that "negative" scores for any particular criteria do not necessarily equate to evidence towards the variant being benign with respect to that criteria, but rather that the criteria may be "unavailable for assessment". At paragraphs 4.12 to 4.15 below we identify a number of limitations in this respect.

4.8 The variants that do not meet the required criteria to be classified as "pathogenic" or "likely pathogenic", and have not been shown to be "benign" or "likely benign", are classified as "variants of unknown significance" (**VUS**). As sufficient information becomes available with newly discovered variants, including reports of additional patients with the same variant and disease, and/or functional validation with a well-established laboratory assay, a VUS can be reclassified as benign or pathogenic.

4.9 Potential clinical actions to be taken based on the genomics analysis of this kindred could include clinical action for Kathleen Folbigg, and indeed we have already recommended Ms Folbigg undertake a thorough cardiac examination. Other action may result from these genetic variants that are not clinical.

B. Classifying gene variants for non-clinical action

4.10 The ACMG standards for assessing pathogenicity are not intended to be applied to research findings. To date, research findings have formed the basis of many of the reported genetic associations with disease, and researchers continue to report their findings of "likely pathogenic" variants on a case-by-case basis without necessarily adhering, or even refering, to the ACMG criteria. This approach is recognised by the

ACMG and, indeed, dealt with in their disclaimer at the start of their consensus paper:

> *These ACMG Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome.*

4.11 As there are no criteria for reporting variants that might form the basis for non-clinical actions, we have elected to provide information regarding variants of unknown significance, which are not clinically actionable, but for which a causal action in relevant pathology cannot be excluded with confidence based on current knowledge. Thus, this report considers and discusses VUS, in addition to "likely pathogenic" or "pathogenic" variants, that could plausibly cause the phenotypes observed in the children.

C. Specific limitations in scoring the Folbigg Children and Kathleen Folbigg

4.12 Craig Folbigg, the father of the Folbigg children, declined to provide DNA and has therefore not been sequenced. This makes it impossible to identify any variant in the children as *de novo*, which decreases the number of criteria that can be used to score a variant and makes it more difficult for any variant to reach a "pathogenic" or "likely pathogenic" designation.

4.13 We have been provided with no suitable photographs of the children, which makes identification of soft dysmorphic features difficult. It is well known that even the best dysmorphology geneticists can miss such features, and that they are often only identified after genetic diagnosis has been established.

4.14 The cardiac assessments of the children are incomplete, which may miss important phenotypes, required for variant classification.

4.15 In the case of Kathleen Folbigg, there are further considerations that make it difficult to identify whether she might harbour "pathogenic" or "likely pathogenic" variants. These additional considerations are as follows:

4.15.1 We still do not have a full cardiac assessment of Ms Folbigg including the tests required to diagnose Long QT Sd (24 hr Holter monitor, exercise test ECG). Accordingly, scoring of variants may underestimate pathogenicity,

since segregation with any putative cardiac disease in the mother (as suggested by her own documented reports of several episodes of syncope) will not be taken into account.

4.15.2 If Ms Folbigg suffers from any type of clinically manifest cardiac condition, or if she is a non-penetrant carrier of a potentially pathogenic *de novo* variant in a known cardiac gene, it would not be possible to assess Ms Folbigg's variants against the *de novo* criteria (including maternity and paternity assessment), because both of Ms Folbigg's parents are deceased and their genomes were not sequenced. This decreases the number of criteria that can be used to score the variants and makes it more difficult for any variant to reach a "pathogenic" (or "likely pathogenic") score.

5. METHODOLOGY

5.1 In this report we examine rare gene variants (minor alleale frequency, **MAF**, <0.005), most of which are "ultra-rare" (MAF <0.0005), that may have contributed to the sudden unexpected deaths of the Folbigg children.

5.2 The variants were identified using next generation sequencing technologies (whole exome sequencing and/or whole genome sequencing).

5.3 Analysis of chromosomal microarray and testing for trinucleotide expansion disorders was not performed as part of this analysis.

5.4 Analysis of the mitochondrial genome was carried out on the samples obtained from AGRF.

5.5 SNV and Indel variants were called using two independent bioinformatic pipelines at ANU and NSW Health. Variants from both pipelines were considered.

5.6 Structural variants were called using Delly and Lumpy tools using the ANU bioinformatic pipeline.

5.7 Information from PubMed links using search terms agreed with by all genetics experts was considered and when appropriate, extracts from such references/links are copied in the report. The terms included:

Arrest OR Arrhythm OR Atrial fibrillation OR Atrioventricular block OR Brugada OR Cardiac OR Cardiomyopathy OR Channelopathy OR Conduction abnormalit* OR Conduction defect OR Conduction disorder OR Congenital heart disease OR Defibrillator OR Epilep* OR Heart OR Heart block OR Hippocamp* OR Hypotonia OR ICV OR ICVD OR Left ventricular noncompaction OR Left ventricular non-compaction OR Myocyte OR Myotoni* OR Periodic paralysis OR Respiratory control OR Resuscitat* OR Seizures OR Sodium channel OR Ventricular fibrillation OR Ventricular tachycardia*

5.8 Additional information considered included all the fields in the raw reports provided by both informatic pipelines (i.e. information on mouse phenotypes present in the Canberra cohort report, links to OMIM, population frequencies, *in silico* predictions of variants' damaging effects).

5.9 With respect to *in silico* predictions of variants' damaging effects, in general, three prediction software tools were considered: **Polyphen2** (scores between 0 to 1, with 1 being most damaging), **Sift** (sores between 0 to 1 with 0 being most damaging) and **CADD** (scores above 12-14 are usually considered damaging).

5.10 External databases including ClinVar and Varsome were consulted for instances of pathogenic variants and previous classifications.

5.11 Additional information that may support a degree of pathogenicity of variants classified as VUS is provided where relevant.

5.12 Samples were not available for orthogonal testing to confirm variants.

6. SUMMARY OF FINDINGS

6.1 No known “pathogenic” or “likely pathogenic” variants in genes that could explain unexpected death were found in 4 out of 4 children.

6.2 Two likely pathogenic (***IDS*** and ***CALM2***), and one borderline VUS or likely pathogenic (***MYH6***), missense novel or ultrarare variants that could contribute to the observed phenotypes were found in the children:

6.2.1 ***IDS***: Found in Patrick, X-linked (mother and sisters are carriers). Likely pathogenic. Previously, a different hemizygous mutation affecting the same codon as the one found in Patrick has been shown to cause Hunter Syndrome, a metabolic disease that can cause seizures and death due to cardiac or respiratory arrest.

6.2.2 ***CALM2***: Likely pathogenic. Found in Sarah and Laura. Heterozygous *CALM2* mutations have been found in several infants with severe forms of long-QT syndrome (**LQTS**) who displayed life-threatening ventricular arrhythmias together with delayed neurodevelopment and epilepsy.

6.2.3 ***MYH6***: Borderline VUS or likely pathogenic (depending on whether mother has cardiac conduction defect). Found in Laura and Caleb. Heterozygous mutations in this gene have been shown to cause sudden cardiac death with incomplete penetrance.

6.3 A number of variants of unknown significance present in all of the deceased children occur in genes considered to have the potential to contribute to disease. Some of these variants occur in the genes: ***KCNQ1, SEMA3A, PRKAG2, DMPK, ADAMTS6, TAB1, SLC12A9.*** When this report was compiled, there was insufficient evidence to conclude the variants are either benign or likely pathogenic.

6.4 A number of variants of unknown significance were present in only some of the deceased children and considered to have the potential to contribute to disease. These include ***MYLK, CACNA1E, JUP***, ***ADSL and NLRP1***. When this report was compiled, there was insufficient evidence to conclude the variants are either benign or likely pathogenic.

6.5 **Figure 1** below sets out an annotated pedigree of the Folbigg family noting the variants of interest for each member of the family, and those variants which are assumed to be present in Craig Folbigg.

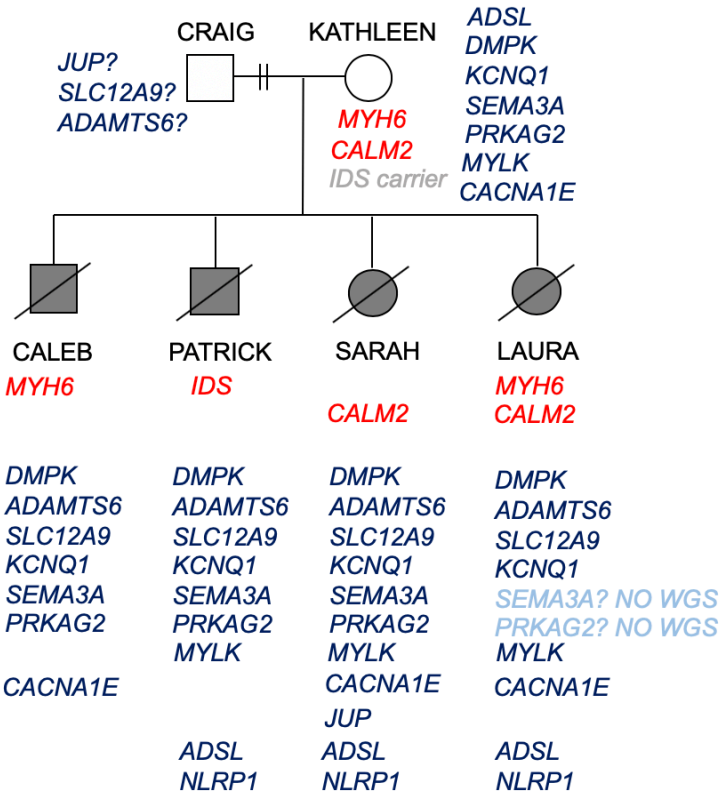


Figure 1 *Genes carrying ultra-rare variants that may contribute to the deaths of the Folbigg children. (**Red** = Likely pathogenic. **Blue** = VUS)*

7. DETAILED REPORT: COMMON CAUSE INVESTIGATION

7.1 The first part of the detailed report describes variants that were detected when conducting a sequencing analysis assuming a common causal variant in all children located in a coding region.

A. Autosomal dominant model

7.2 There were 22 single nucleotide variants (SNVs or indels occurring at a frequency <0.001% according to both ExAC and gNOMAD) present in all 4 children in which the relevant region was covered. None of these were classified as pathogenic or likely pathogenic. We identified four variants of uncertain significance in all four siblings that are predicted to affect proteins in potentially relevant pathways. These are *DMPK, ADAMTS6, SLC12A9* and *TAB1*.

7.3 ***DMPK*** DM1 Protein Kinase

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal	p.Gly31Arg	gnomAD: 0.000047 ExAC: 0.000032	Polyphen 0.005; Benign SIFT 0.12; Tolerated CADD 8.097	Het	Caleb Patrick Sarah Laura	VUS

7.3.1 *DMPK* mutations (repeat expansions) cause Brugada Syndrome and cardiac death (Sabovic et al., 2003; Wahbi et al., 2013).

7.3.2 Repeat expansions in this gene cause dystrophia myotonica type I (DM1). A case was described where a *DMPK* expansion allele contributed to Brugada syndrome in a patient without dystrophia myotonica (Pambrun et al., 2014):

> *The proband, a 15-year-old female, was a survivor of out-of-hospital cardiac arrest with ventricular fibrillation. She combined a DMPK CTG expansion from the father's side and an SCN5A mutation (S910L) from the mother's side. Surprisingly, in the father, a DM1 patient without SCN5A mutation, ajmaline also unmasked a Brugada phenotype. Furthermore, association of both genetic abnormalities in the proband exacerbated the response to ajmaline with a massive conduction defect.*

7.4 Mice homozygous for a null mutation exhibit abnormal sodium channel gating in cardiac myocytes, cardiac conduction defects, and late-onset progressive skeletal myopathy. Suggests severe mutations can first cause cardiac conduction defects and later dystrophia myotonica (Reddy et al., 1996; Jansen et al., 1996).

7.5 ***ADAMTS6*** ADAM Metallopeptidase With Thrombospondin Type 1 Motif 6

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Unknown[3]	p.Gly592Ala	gnomAD: 0.000162 ExAC: 0.000033	Polyphen 0.403; Benign Sift 0.5; Tolerated CADD 23.1; Damaging	Het	Caleb Patrick Sarah Laura	VUS

7.5.1 Mutation in TSP type-1 1 domain; G residue forms part of the disulphide bond

7.5.2 "Exome-chip meta-analysis identifies novel loci associated with cardiac conduction, including *ADAMTS6*" (Prins et al., 2018):

> *Functional studies: In vitro validation analysis shows that the QRS-associated variants lead to impaired ADAMTS6 secretion and loss-of function analysis in mice demonstrates a previously unappreciated role for ADAMTS6 in connexin 43 gap junction expression, which is essential for myocardial conduction.*

7.6 ***SLC12A9*** Solute Carrier Family 12 Member 9

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Unknown	p.Pro876Leu	gnomAD: 0.000032 ExAC: N/A	Polyphen 0.997; Probably damaging SIFT 0; Deleterious CADD 18.7	Het	Caleb Patrick Sarah Laura	VUS

7.6.1 Common variants in *SLC12A9* have been associated with modulation of cardiac ECG parameters (Eijgelsheim et al., 2010).

[3] "Unknown" in this context means that the mode of inheritance was likely paternal because the mutation was not seen in the mother's WGS, but it may have been *de novo* (i.e. a result of paternal or maternal mosaicism) as we did not have Craig Folbigg's DNA to confirm.

7.7 ***TAB1*** TGF-Beta Activated Kinase 1 (MAP3K7) Binding Protein 1

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Unknown	p.Arg489Cys	gnomAD: 0.00007 ExAC: 0.000140	Polyphen 0.985; Probably damaging SIFT 0; Deleterious CADD 35	Het	Caleb Patrick Sarah Laura	VUS

7.7.1 *TAB1* (MASP3K7) encodes TGF-beta activated kinase 1, which mediates various intracellular signaling pathways relevant to numerous biological processes, including inflammation and organogenesis *(Wade et al., 2016; Le Goff et al., 2016).*

7.7.2 The variant identified has a very low population prevalence (MAF: 0.00007 Gnomad), and in silico scores support pathogenicity (PP2 0.985, CADD 35). As noted in the table, all four siblings were heterozygous for the variant. So far, however, no diseases have been attributed to rare mutations in *TAB1*.

B. Autosomal recessive model:

7.8 The cohort was analysed for homozygous or compound heterozygous mutations including at least one rare allele. No candidate variants were identified irrespective of segregation.

8. DETAILED REPORT: SIDS PHENOTYPE INVESTIGATION

8.1 The second part of the detailed report describes variants that were detected when conducting a sequencing analysis assuming SIDS was the prevailing phenotype. This analysis examined mutations shared across all four children in non-coding regions and mutations in coding regions that were not necessarily shared across the children.

8.2 349 genes were identified from the published literature after a PubMed search using a consensus list of search terms arrived at by the genomicists involved in the analysis of this case (see Appendix 1). From this candidate gene list, the following variant alleles were identified after filtering for rarity plus *in silico* scores predicting damage.

A. Structural variants present in all 4 children

8.3 To date, more than 50% of monogenic diseases remain undiagnosed after WES (Noll et al., 2016; Monlong et al., 2018). It is thought that larger structural variants, including exonic, intronic, and intergenic insertions, deletions, duplications and translocations explain a large fraction of remaining undiagnosed cases. Variants in non-coding regions often harbour cryptic intronic splice sites, or affect neighbouring gene enhancers or promoters. This is a relatively novel area of research. It is expected that these variants will be gradually reported over the next decade.

8.4 We still lack bioinformatic tools that can predict the damaging potential of structural variants, particularly when these are found in non-coding regions of the genome. Validation of their pathogenicity usually involves expression analysis, using a range of RNA-expression technologies.

8.5 Four rare structural variants were identified in candidate genes, in all four Folbigg children (or all three children where Laura's sequence data had no coverage in the region as she was only sequenced via WES). They all occur in intronic regions, and none are classified as pathogenic. They are *KCNQ1*, *SEMA3A*, *PRKAG2* and *FHOD3*. Details on the first three are provided below

8.6 ***KCNQ1*** Potassium Voltage-Gated Channel Subfamily Q Member 1

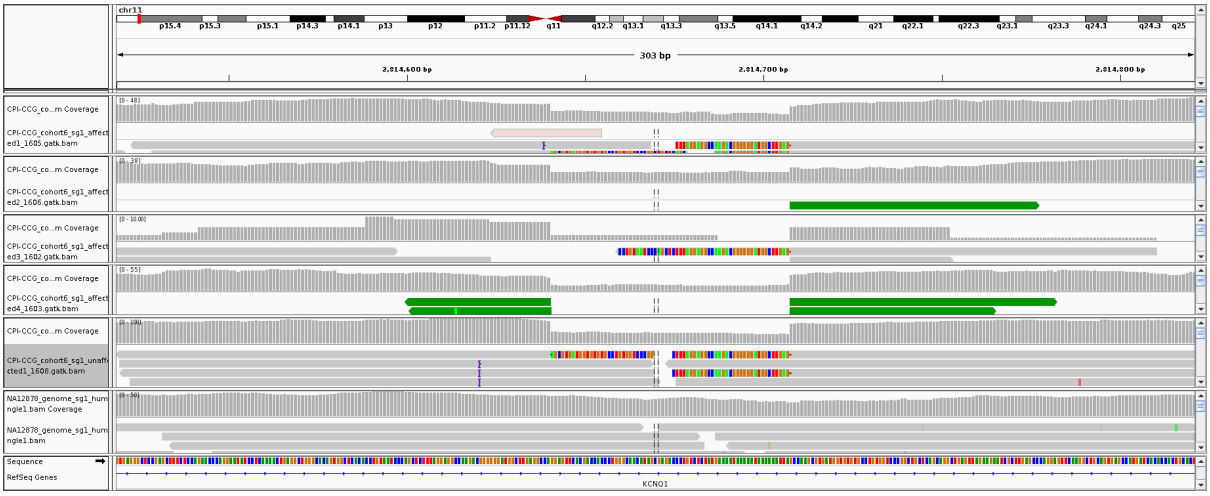
Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal	72 bp intronic deletion	Novel[4]	N/A	Het	Caleb Patrick Sarah Laura	VUS

8.6.1 *KCNQ1* mutations are present in patients with a family history of lethal cardiac arrhythmias and sudden death. Long QT Syndrome (**LQTS**) is a well-characterised cardiac ion channelopathy, which causes syncope and sudden death. LQT1, due to mutations of *KCNQ1* (KVLQT1), is the most common form of LQTS. Novel mutations in *KCNQ1* are frequently still being described (Chen et al., 2003; Nagasawa et al., 2018; Liin, Barro-Soria, & Larsson, 2015).

[4] Novel refers to the fact the variant has never before been reported in the research literature.

8.6.2 A study describing the genotype-phenotype characteristics in 10 families with mutations of KCNQ1 described 40% of patients with mutations and cardiac events had normal to borderline QTc (< or = 0.46 s) and in 33% of sudden deaths (SD) caused by KCNQ1 mutations, SD occurred as the first symptom. Findings emphasize (1) reduced penetrance of QTc and symptoms, resulting in diagnostic challenges, (2) the problem of sudden death as the first symptom (33% of those who died), and (3) genetic testing is important for identification of gene carriers with reduced penetrance (Chen et al., 2003).

8.6.3 Visual representation of the *KCNQ1* deletion in the 4 children and mother using IGV viewer:



SEMA3A Semaphorin 3A

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal	320 bp intronic insertion	Novel	N/A	Het	Caleb Patrick Sarah[5]	VUS

8.6.4 *SEMA3A* – intronic insertion. Novel, not described before in databases of variation. Heterozygous mutations in SEMA3A cause Brugada Sd. Clinical presentations of Brugada Sd include sudden infant death syndrome (SIDS; death of a child during the first year of life without an identifiable cause) and the sudden unexpected nocturnal death syndrome (SUNDS). It is caused by various conduction defects including ST segment abnormalities with high propensity for ventricular arrhythmias, first-degree AV block, intraventricular

[5] No coverage by WES of the region in Laura.

conduction delay, right bundle branch block, and sick sinus syndrome. (Brugada et al., 2005)

PRKAG2 Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 2

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal	187 bp intronic insertion	Novel	N/A	Het	Caleb Patrick Sarah[6]	VUS

8.6.5 *PRKAG2* intronic insertion. Novel, not described in databases of genetic variation. The *PRKAG2* gene regulates the γ2 subunit of the adenosine monophosphate (AMP) dependent protein kinase. *PRKAG2* mutations cause cardiac specific non-lysosomal glycogenosis and have been associated with the development of atrioventricular (AV) accessory pathways, cardiac hypertrophy, and conduction system abnormalities (ventricular pre-excitation and supraventricular arrhythmias) (Aggarwal et al., 2015).

8.6.6 PRKAG2 syndrome (PS) is a rare, early-onset autosomal dominant inherited disease. PS can show different expressivity both of ventricular hypertrophy and arrhythmic features, ranging from an asymptomatic condition to sudden cardiac death (SCD) (Porto et al., 2016).

B. SNVs and small indels present in 1 or more children

8.7 Several variants were found in candidate genes of one or more of the Folbigg children. Of those, two were likely pathogenic or borderline VUS-likely pathogenic:

8.8 ***CALM2*** Calmodulin 2

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal	p.Gly114Arg	Novel	Polyphen 0.992; Probably Damaging SIFT 0.01; Deleterious CADD 22.1	Het	Sarah Laura	**Likely pathogenic**

8.8.1 Several infants with severe forms of LQTS who displayed life-threatening ventricular arrhythmias together with delayed neurodevelopment and epilepsy

[6] No coverage by WES of the region in Laura.

were found to have heterozygous mutations in either this gene or another member of the calmodulin gene family (Crotti et al., 2013). Mutations in this gene have also been identified in patients with less severe forms of LQTS ((Makita et al., 2014; Schwartz et al., 2013; Fernandez-Falgueras et al., 2017; Jensen, et al., 2018; Jimenez-Jaimez et al., 2016).

8.8.2 *Scoring criteria provided below:* 1(2) moderate P + 4 supporting P.

Pathogenicity Criterion	***CALM2*, p.114G>R**	
A. VERY STRONG (VSP)		
PVS1. Null variant	No	not assessed, predicted not null
B. STRONG (SP)		
PS1. Same aa change previously pathogenic	No	
PS2. De novo (confirmed parentity)	No	
PS3. Established function test - damaging	N/A	
PS4. Prevalence in patients > controls	No	
C. MODERATE (MP)		
PM1. Variant hotspot, functional domain	Yes	https://www.uniprot.org/uniprot/P0DP24 aa 81 – 116 EF-hand 3 domain
PM2. Absent, rare in healthy databases	Yes	Novel
PM3. For recessive, phase trans	N/A	
PM4. Change in protein length	No	
PM5. Same residue, different aa change pathogenic	No	
PM6. Assumed de novo (w/o parentity)	No	
D. SUPPORTING (SP)		
PP1. Co-segregation in the family	No/Yes	2/4 affected, 3 affected if mother has Long QT Sd→ weak supporting evidence (Nykamp et al., 2017).
PP2. Low rate of benign missense variation and in which missense variants are a common mechanism of disease	Yes	No missense benign variants described. Most missense variants described cause Long QT Sd and death in infancy. pLI=0.89
PP3. In silico damaging, multiple lines	Yes	
PP4. Phenotype specific for disease	Yes	SIDS
PP5. Reported pathogenic, w/o evidence provided	No	
TOTAL SCORE	1(2) moderate P + 4 supporting P	
INTERPRETATION	Likely pathogenic	

a. PP1. Weak segregation requires a minimum of three individuals with the variant with a dominant condition (Nykamp et al., 2017).

b. If the variant lies in a “hotspot” or functional domain, segregation in the family does not need to be met. Gly114 lies in the EF-hand 3.

The EF-hand motif is among the most common of structural motifs in animal cells (Henikoff et al., 1997). These motifs are organized into structural units/domains containing two or more EF hands that form highly stable helical bundles (Nelson & Chazin, 1998). EF-hand proteins can function as Ca2+ sensors, which transduce Ca2+ signals, or Ca2+ signal modulators. Calmodulin (CaM) is the most important Ca2+ signal transducer in eukaryotic cells. CaM is one of the most conserved proteins in evolution, with a sequence of 148 amino acids that is identical in all vertebrates (the first methionine is lost in the mature protein). It is encoded by three independent genes (CALM1-3) in humans, all translating identical CaM sequences. It is composed of four Ca2+-binding EF-hands, and each contains a helix-loop-helix motif with a central acidic loop of 12 residues for Ca2+ coordination via at least six oxygen atoms. Gly114 lies in the EF-hand 3, which is part of the C-lobe.

Except one (N54I), all other seventeen amino acid replacements so far described are located in the C-lobe and connected with different cardiac dysfunctions, some associated with sudden cardiac arrest following exercise or emotion. Some mutations have been structurally analyzed, and found to cause variable conformational changes of the whole C-lobe (Urrutia et al., 2019).

8.8.3 So far, only 30 patients have been described carrying *CALM* mutations, of which 20 were *de novo* mutations and three inherited (N54I, F90L and A103V) (Kotta et al., 2018). At present it cannot be established if the mutation in the mother occurred *de novo*. The mother may or may not be affected with Long QT Sd, but the diagnosis is plausible based on a clinical history of multiple syncope episodes. Interpretation of this variant will be influenced by additional cardiac testing, and at present the PS2 criterion cannot be determined.

8.9 ***MYH6*** Myosin Heavy Chain 6

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal	p.Pro82Ser	gnomAD:N/A ExAC: 0.000008	Polyphen 0.519; Possibly Damaging SIFT 0.01; Deleterious CADD 26.4	Het	Caleb Laura	**Likely pathogenic** (or VUS)

8.9.1 The variant in *MYH6* is ultra-rare and is not listed in ClinVar or Varsome. Other mutations in *MYH6* have been associated with cardiac disease, including atrial septal defects, hypertrophic cardiomyopathy and sinus node dysfunction, with notably, with early onset, recurrent, and fatal arrhythmias (Lam et al., 2015).

8.9.2 Scoring is borderline between VUS or likely pathogenic.

8.9.3 The distinction between VUS or likely pathogenic would rely on PM1. Namely, whether amino acid position 82 is in a "hotspot" or functional domain. This amino acid is immediately adjacent to the SH3 domain which is critical for protein interactions and ends with Pro in position 81. Thus, this criterion is subject to interpretation.

8.9.4 Two out of 4 affected children harbouring the mutation can be considered as segregating (the other two could be considered phenocopies, or alternative explanations). If the mother is found to have a pathological cardiac phenotype (i.e. the reported history of syncope is found to be of cardiac origin), then this would obviously result in reclassification of the variant based on segregation.

Pathogenicity Criterion	***MYH6*, p.82P>S**	
E. VERY STRONG (VSP)		
PVS1. Null variant	No	not assessed, predicted not null
F. STRONG (SP)		
PS1. Same aa change previously pathogenic	No	
PS2. De novo (confirmed parentity)	No	
PS3. Established function test - damaging	N/A	
PS4. Prevalence in patients > controls	No	
G. MODERATE (MP)		
PM1. Variant hotspot, functional domain	Yes?	SH3 domain 32-81
PM2. Absent, rare in healthy databases	Yes	MAF = 0.000008, 0.00002/3
PM3. For recessive, phase trans	N/A	
PM4. Change in protein length	No	
PM5. Same residue, different aa change pathogenic	No	2014: Pro82Leu assessed as VUS
PM6. Assumed de novo (w/o parentity)	No	
H. SUPPORTING (SP)		

PP1. Co-segregation in the family	Yes	2/4 affected, parent not affected??
PP2. Low benign variant frequency	No	
PP3. In silico damaging, multiple lines	Yes	
PP4. Phenotype specific for disease	Yes	SIDS
PP5. Reported pathogenic, w/o evidence provided	No	
TOTAL SCORE	2(1) moderate P + 3 supporting P	
INTERPRETATION	likely pathogenic or VUS	

8.10 Additional rare variants of unknown significance found in candidate genes in the Folbigg children are shown in the table below:

Gene (NCBI Reference Sequence)	**Variant**	**Zygosity**	**Clinical Significance**	**Associated Disorder(s) (inheritance)**	**Carriers**
MYPN (NM_0032578.2)	c.3335C>A (p.Arg489Cys)	Het	Uncertain	Dilated cardiomyopathy (AR)	Kat Cal Sar
CHRNB2	(p.Pro876Leu)	Het	Uncertain	Nocturnal frontal lobe epilepsy (AD)	Kat
CACNA1E	p.Ala10Asp	Het	Uncertain	Epileptic encephalopathy, early infantile (AD)	Sar, Lau and Cal
CLCN2 (NM_004366.4)	c.1930C>T (pArg644Cys)	Het	Uncertain	Juvenile myoclonic epilepsy (AD); leukoencephalopathy with ataxia (AR)	Cal, Lau
CTNNA3 (NM_0013266.3)	c.1900G>A (p.Glu643Lys)	Het	Uncertain	Arrhythmogenic right ventricular dysplasia 13 (AD)	Cal, Sar
ADSL	p.Gly4Val	Het	Uncertain	Adenylosuccinase deficiency (AR) (Developmental delay, epilepsy. Can cause severe encephalopathy with brain atrophy and hypomyelination)	Sar, Pat and Lau
JUP NM_002230.2)	c. 578T>C (p.Met194Thr)	Het	Uncertain	Arrhythmogenic right ventricular cardiomyopathy with keratosis (AD)	Sar
GLI3	p.Asn1416Ile	Het	Uncertain	Hypothalamic Hamartoma with Gelastic Epilepsy	Pat

9. DETAILED REPORT: PATRICK'S PHENOTYPE

9.1 The third part of the detailed report describes variants that were detected when conducting a sequencing analysis specifically to address Patrick's phenotype, namely mutations that may explain respiratory arrest and seizures.

9.2 We examined any variants that were rare homozygous, X-linked or compound heterozygous. This revealed a likely pathogenic X-linked variant in *IDS*.

9.3 ***IDS*** Iduronate 2-Sulfatase

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Children	Classification
Maternal (X-linked)	p.Pro467Ala	Novel gnomAD:N/A ExAC: N/A	Polyphen 0.987; Probably Damaging SIFT 0 Deleterious CADD 24.8	X-linked	Sarah & Laura carriers Patrick YHem	**Likely Pathogenic**

9.3.1 Iduronate 2 Sulfatase (**IDS**) is X-linked and the cause of Hunter Sd, which is a metabolic disease. Death is usually caused by respiratory or cardiac failure, see below.

Pathogenicity Criterion	***IDS*, p.467P>A**	
I. VERY STRONG (VSP)		
PVS1. Null variant	No	not assessed, predicted not null
J. STRONG (SP)		
PS1. Same aa change previously pathogenic	No	
PS2. De novo (confirmed parentity)	No	
PS3. Established function test - damaging	N/A	
PS4. Prevalence in patients > controls	No	
K. MODERATE (MP)		
PM1. Variant hotspot, functional domain	No	
PM2. Absent, rare in healthy databases	Yes	novel
PM3. For recessive, phase trans	N/A	
PM4. Change in protein length	No	
PM5. Same residue, different aa change pathogenic	Yes	467P>R: 1 paper (1 patient), not in ClinVar; 467P>L: 2 papers (3 patients, 1 severe), not in ClinVar
PM6. Assumed de novo (w/o parentity)	No	
L. SUPPORTING (SP)		
PP1. Co-segregation in the family	Yes	CPI501 is the only hemizygote
PP2. Low benign variant frequency	Yes	pLI=0.98 (see below)
PP3. In silico damaging, multiple lines	Yes	Paper indicating severe misfolding
PP4. Phenotype specific for disease	Yes	Early death under 1 year has been

		described
PP5. Reported pathogenic, w/o evidence provided	No	Called pathogenic before, not in ClinVar, no criteria provided
TOTAL SCORE	2 moderate P + 4 supporting	
INTERPRETATION	Likely Pathogenic (see consideration below)	

9.3.2 PM5: The mutation identified here is unique, but affects a codon previously implicated in disease. It is reasonable to consider the 3 reported Hunter Sd cases with IDS 467P>L and 1 with IDS 467P>R as pathogenic. Pathogenicity assessment in these reports was based on computational predictions, compatibility with phenotype, but not using a formal framework, not in ClinVar. If these variants are not pathogenic, the variant can be classified as PP5. In either case there would be enough evidence for "likely pathogenic".

a. Mutated proline-467 to leucine found in 2 cases of Hunter Sd (Froissart et al., 1998; Moreira da Silva et al., 2001), one severe: https://web.expasy.org/variant_pages/VAR_026956.html

b. P467A. Mutated proline-467 to arginine in 1 patient with no enzyme activity (patient no. 52 in the supplement of the reference (Pollard, Jones, & Wood, 2013)).

c. Crystal structure of IDS and computational analysis indicate that the amino acid proline in position 467 belongs to the SD2 subdomain of the enzyme and changes of this amino acid to either leucine or arginine (previously described pathogenic variants) leads to severe protein misfolding, consistent with the previous report of loss of enzyme activity in the arginine variant (see supplement of the reference : (Demydchuk et al., 2017).

9.3.3 X-linked mode of inheritance (a hemizygous male manifests the condition, a carrier female is asymptomatic or mild symptoms). Present in Patrick (not present in Caleb). Mother and both sisters are carriers.

9.3.4 Death is usually caused by respiratory or cardiac failure and may occur at a young age under 5 years in about 5% of cases, and as early as 6 months of age. Airways problems, including choking, cardiac causes and neurological problems are listed causes of death in about a half of the children with fatal

outcome in very young age (under 10 years and under 5 years). Respiratory and cardiac failure are the leading cause of death in older patients as well (Jones et al., 2009; Broomfield et al., 2017; Lin et al., 2016).

9.3.5 Patrick was hospitalised after developing what was thought to be a non-infectious encephalopathy, causing seizures. Differential diagnosis at the time included metabolic disorders. Several metabolic / lysosomal diseases were screened for. Mucopolysaccharides were tested in the urine of Patrick and reported normal. The specific types of mucopolysaccharides tested were not detailed in the report. The current approach to the diagnosis of MPS II depends on demonstrating an absence or reduced iduronate 2-sulfatase (I2S) enzyme activity in white cells, fibroblasts, or plasma. Most affected males have no detectable I2S activity using the artificial substrate. Identification of a hemizygous *IDS* pathogenic variant by molecular genetic testing confirms the diagnosis of MPS II in a male proband and may be useful in persons with an unusual phenotype or a phenotype that does not match the results of GAG analysis.

9.3.6 Regarding the absence of coarse facial features in Patrick:

> *The appearance of newborns with MPS II is normal. Coarsening of facial features – the result of macroglossia, prominent supraorbital ridges, a broad nose, a broad nasal bridge, and deposition of GAG in the soft tissues of the face resulting in large rounded cheeks and thick lips – generally manifests between ages 18 months and four years in the early progressive form and about two years later for those with the slowly progressive form. Some develop ivory-colored skin lesions on the upper back and sides of the upper arms, pathognomonic of MPS II (Tylki-Szymanska, 2014).*

9.4 No mutations were present in Patrick in genes associated with Rett Sd.

9.4.1 A differential diagnosis of Rett Sd was suggested by neurologists in view of Patrick's clinical presentation.

9.4.2 No rare variants in *MECP2*, *FOXOG1* or *CDKL5* were found (coverage of exon 1 of *CDKL5* was inadequate).

9.5 Ultra-rare mutations in Patrick in epileptogenic and metabolism genes include missense mutations in ***ADSL***, ***LARGE2*** and ***GLI3*** (Appendix 2).

9.6 No mutations were found in genes known to cause monoamine neurotransmitter disorders (*GCH1, PTC, SRP, DHPR, PCBD1, TH, DDC, PNPO, PITX3, MAOA, MAOB, NBD, DBH, SLC18A2, SLC6A3*).

9.7 Mutations in immune genes were investigated. There were two novel / ultrarare missense variants of unknown significance in genes involved in innate immune responses or responses to bacteria:

9.7.1 ***NLRP1***

a. NLRP1 deficiency in humans causes autoinflammatory diseases: Autoinflammation with arthritis and dyskeratosis (AD, AR).

b. Death in mice lacking NLRP1 is associated with myocarditis (Masters et al., 2012).

Inheritance	Variant	Frequen-cy	Damage Prediction	Zygo sity	Children	Classification
Unknown (presumed paternal)	p.Gly881Arg	Novel	Polyphen 0.985 Probably damaging SIFT 0.01 Deleterious CADD 7.6	Het	Pat Laura Sarah	VUS

9.7.2 ***PTPN13***

a. PTPN13 regulates STAT/cytokine signalling; PTPN13 deficient mice are more resistant to bacterial lung infection (Nakahira, 2007).

Inheritance	Variant	Frequency	Damage Prediction	Zygosity	Carriers	Classification
Mother	p.R332W	0.00005	Polyphen 0.931 Probably Damaging SIFT 0.02 Deleterious CADD 29.4	Het	All children Mother	VUS

10. FURTHER INVESTIGATION NEEDED

10.1 Caveats to the interpretation of the WES or WGS sequence data of the Folbigg children include the absence of paternal DNA (from Craig Folbigg), an incomplete family history, and incomplete cardiac assessment of Kathleen Folbigg.

10.2 Further investigations should include:

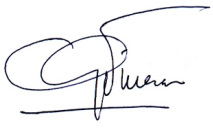
10.2.1 RNA sequence of heart or brain tissue samples of the children if available and adequately preserved. This form of sequencing could provide insight as to the pathogenicity of the structural variants in introns (*PRKAG2, SEMA3A, KCNQ1*). It could be performed in 3-6 weeks.

10.2.2 Re-analysis of SNP arrays to include not just known pathogenic changes (as the current form of the reports), but potentially relevant variants determined from candidate gene lists.

10.2.3 Functional analysis for iduronate 2-sulfatase activity in Patrick's fibroblasts to help diagnose Hunter Sd.

10.3 With respect to any clinical action required, Ms Folbigg should undertake a full cardiac assessment under the direction of a cardiac electrophysiologist to test for Long QT Syndrome. This would likely involve a 24 hr Holter monitor and an exercise test ECG.

10.4 Consultation with a genetic cardiologist / cardiac geneticist.



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Appendix 1. Candidate Gene List

AARS2
ABCC9
ACAD9
ACADL
ACADM
ACADS
ACADVL
ACAT1
ACHE
ACTC1
ACTN2
ADCYAP1
ADORA1
ADORA2A
ADRA1A
AGRN
AKAP9
ALDH7A1
ALDOB
ALG14
ALG2
ANK2
ANK3
ANKRD1
APOE
APOPT1
AQP4
ARHGEF9
ARX
ASAH1
ASCL1
ASL
ASS1
AUH
BAG3
BDNF
BMP2
BMPR2
BTD
C4A
C4B
CACNA1A
CACNA1B
CACNA1C
CACNA1D
CACNA1E
CACNA1F
CACNA1G
CACNA1H
CACNA1I
CACNA1S
CACNA2D1
CACNA2D2
CACNA2D3
CACNA2D4
CACNB2
CACNB4
CALM1
CALM2
CALM3
CALR3
CARS2
CASP3
CASQ2
CASR
CAV3
CBL
CCKBR
CDKL5
CHAT
CHD2
CHRM2
CHRM3
CHRNA1
CHRNA2
CHRNA4
CHRNA7
CHRNB1
CHRNB2
CHRNB4
CHRND
CHRNE
CHRNG
CLCN2
CLCNKB
CLN8
CLU
CNTN1
CNTN2
CNTNAP2
COL13A1
COL3A1
COLQ
COMT
COQ2
COX10
COX14
COX20
COX6B1
COX8A
CPA6
CPS1
CPT1A
CPT2
CRYAB
CSRP3
CSTB
CTF1
CTNNA3
CXCL8
CYP1A1
CYP21A2
CYP3A4
CYP3A43
CYP3A5
CYP3A7
DDC
DEPDC5
DES
DLD
DMD
DMPK
DNASE1
DNASE1L3
DNM1
DOCK7
DOK7
DPAGT1
DPP6
DRD2
DSC2
DSG2
DSP
DTNA
ECE1
EDN1
EDN3
EFEMP2
EFHC1
EGR2
EMD
EN1
EPM2A
ETFA
ETFB
ETFDH
EYA4
FAH
FASTKD2
FBN1
FBN2
FBP1
FEV
FHL2
FHOD3
FKTN
FLNC
FMO3
FOXRED1
FXN
G6PC
GAA
GABRA1
GABRB3
GABRD
GABRG2
GATA4
GATAD1
GCDH
GCK
GDNF
GFPT1
GJA1
GJA5
GK
GLA
GLDC
GLRA1
GLRA3
GLRB
GLUD1
GNAO1
GNB3
GOSR2
GPD1L
GRIN1
GRIN2A
GRIN2B
GSTT1
HADH
HADHA
HADHB
HCN1
HCN2
HCN4
HLA-DRB1
HLA-DRB3
HLA-DRB5
HLCS
HMGCL
HMGCS2
HRH1
HSD17B10
HSPD1

HTR1A
HTR2A
HTR2B
HTR3A
HTR4
HTR7
IER3IP1
IGHMBP2
IL10
IL13
IL1A
IL1B
IL1R1
IL1RN
IL6
IL6R
ILK
IVD
JAG1
JPH2
JUP
KCNA1
KCNA5
KCNB1
KCND3
KCNE1
KCNE2
KCNE3
KCNE4
KCNE5
KCNH2
KCNJ18
KCNJ2
KCNJ5
KCNJ8
KCNMA1
KCNQ1
KCNQ2
KCNT1
KCTD7
L2HGDH
LAMA4
LAMP2
LDB3
LGI1
LMNA
LMX1B
LRP4
MAOA
MAOB
MAP2
MAP2K1
MBL2
ME2
MEF2C
MIB1
MPZ
MTO1
MUSK
MUT
MYBPC3
MYH11
MYH6
MYH7
MYL2
MYL3
MYLK
MYLK2
MYOM1
MYOT
MYOZ2
MYPN
NDUFA1
NDUFA11
NDUFAF1
NDUFAF2
NDUFAF3
NDUFAF4
NDUFAF5
NDUFB3
NDUFB9
NDUFS1
NDUFS2
NDUFS3
NDUFS4
NDUFS6
NDUFV1
NDUFV2
NEBL
NECAP1
NEXN
NF1
NHLRC1
NKX2-5
NOS1AP
NOTCH1
NPPA
NTRK2
NUBPL
OPRD1
OPRM1
OTC
P2RX2
P2RY1
PAFAH1B1
PCCA
PCDH19
PCK2
PDLIM3
PET100
PHOX2A
PHOX2B
PKP2
PLCB1
PLN
PNKP
PPARG
PRDM16
PRICKLE1
PRICKLE2
PRKAG2
PRRT2
PSEN1
PSEN2
PTPN11
RAF1
RANGRF
RAPSN
RBM20
RET
RIT1
RNASEH2B
RYR2
SCARB2
SCN10A
SCN11A
SCN1A
SCN1B
SCN2A
SCN2B
SCN3A
SCN3B
SCN4A
SCN4B
SCN5A
SCN8A
SCN9A
SCO1
SDHA
SEMA3A
SGCB
SGCD
SLC12A3
SLC13A5
SLC17A7
SLC22A5
SLC25A20
SLC25A22
SLC2A1
SLC37A4
SLC6A1
SLC6A4
SLC6A5
SLC7A7
SLC9A3
SLC9A3R1
SLMAP
SNAP25
SNIP1
SNTA1
SPTAN1
SRPX2
SST
SSTR2
SSTR3
SSTR4
ST3GAL3
ST3GAL5
STRADA
STXBP1
SULT1A1
SYN1
SYNGAP1
SYT2
SZT2
TAC1
TACO1
TACR1
TACR2
TAZ
TBC1D24
TBX1
TBX5
TCAP
TGFB3
TGFBR1
TH
THBD
TLX3
TMEM43
TMPO
TNF
TNK2
TNNC1
TNNI3
TNNT2
TP63
TPH2
TPM1
TRDN
TREX1
TRHR
TRPM4
TSPYL1
TTN
TTR
TXNRD2
VCL
VEGFA
ZFPM

Appendix 2. Additional metabolism/epilepsy variants (VUS)

11.1.1 ***ADSL***

Inheritance	Nature of Variant	Frequency of Occurrence	Damage Prediction	Zygosity	Children	Clinical significance
From mother	p.Gly4Val	gnomAD: 0.000065 ExAC: 0.000043	Polyphen 0 Benign SIFT 0.15 Tolerated CADD 3.946	Het	Sar, Pat and Lau	VUS

a. Present in Sarah, Patrick and Laura. Inherited from mother.

b. G4→V, no predictions of damage, ultra-rare. Variants in aminoacids 2 and 3 cause severe disease. Reported in ClinVar, VUS, No criteria provided.

c. Adenylosuccinate lyase (ADSL) deficiency is an autosomal recessive disorder of the purine synthesis which results in accumulation of succinylpurines (succinyladenosine (S-Ado) and succinylamino-imidazole carboxamide riboside (SAICAr)) in body fluids. Patients present developmental delay, often accompanied by epilepsy and autistic spectrum disorders. Can cause severe encephalopathy with brain atrophy and hypomyelination:

d. https://www.ncbi.nlm.nih.gov/pubmed/20054783

11.1.2 ***LARGE2***

Inheritance	Nature of Variant	Frequency of Occurrence	Damage Prediction	Zygosity	Children	Clinical significance
From mother	p.Gln716His	gnomAD: 0.000017 ExAC:	Polyphen 0 Benign	Het	Pat	VUS

Professor Carola G. Vinuesa LMS (MBBS), PhD, FFSc (RCPA), FAA
Elizabeth Blackburn NHMRC Principal Research Fellow
Co-Director, Centre for Personalised Immunology (NHMRC CRE)
John Curtin School of Medical Research | The Australian National University | 131 Garran Rd, Acton ACT 2601

		0.000032	SIFT 0.1 Tolerated CADD 1.731			

a. Present only in Patrick (inherited from mother). Q716→H, No predictions of damage. A glycosyltransferase thought to be involved in the maturation of alpha-dystroglycan (alpha-DG). Defects in LARGE2 cause dystroglycanopathies.

b. LARGE2 supported the maturation of alpha-DG more effectively than LARGE and exerted its activity in addition to LARGE. Using deletion mutants of alpha-DG, Fujimura et al. (2005) found that LARGE and/or LARGE2 facilitate the functional glycosylation of the mucin-like domain of alpha-DG. Brockington et al. (2005) showed that GYLTL1B and LARGE localized to the Golgi apparatus and both stimulated alpha-dystroglycan hyperglycosylation and increased its affinity for laminin binding. The authors suggested that GYLTL1B may be a candidate gene for muscular dystrophy, and that its overexpression could compensate for the deficiency of both LARGE and other glycosyltransferases.

11.1.3 ***GLI3***

Inheritance	Nature of Variant	Frequency of Occurrence	Damage Prediction	Zygosity	Children	Clinical significance
Not from mother, presumably from father	p.Asn1416Ile	gnomAD: 0.000058 ExAC: 0.000032	Polyphen 0.35 Benign SIFT 0.09 Tolerated CADD 22.7	Het	Pat	VUS

a. Present only in Patrick (not in mother). Ultra-rare. N1416→I, 1/3 predicted damaging. CADD=23. Causes Hypothalamic Hamartoma with Gelastic Epilepsy.

Carola G. Vinuesa

Professor of Immunology, Department of Immunology and Infectious Disease, John Curtin School of Medical Research, The Australian National University
Director, Centre for Personalised Immunology, NHMRC Centre for Research Excellence
Director, China-Australia Centre for Personalised Immunology, Shanghai Renji Hospital, JiaoTong University.
Genomics Consultant, Canberra Clinical Genomics, ACT Health – ANU Clinical Genomics Diagnostic Service.

Qualifications

LMS (MBBS) Licenciate in Medicine and Surgery, Univ. Autónoma of Madrid (Spain) 1993.
DRCOG Diploma of the Royal College of Obstetricians and Gynaecologists U.K, 1995.
MSc Master in Science (Immunology). University of Birmingham, UK, 1997.
PhD Doctor of Philosophy (Immunogenetics). University of Birmingham, UK, 2000.
FFA Fellow of The Australian Academy of Science. 2015
FFSc (RCPA) Fellow of the Faculty of Science, Royal College of Pathologists of Australasia, 2019.

Previous positions including Competitive Fellowship Awards

1993-1994; General Surgery & Medicine Resident, Wirral Hospitals NHS Trust, UK
1994-1996 Paediatrics, Obs&Gynae, Geriatrics Resident; Hereford Hospitals, UK
1996- HIV & Genitourinary Medicine Resident. Whittall Street Clinic, Birmingham, UK.
1997-2001 MRC Clinical Research Fellowship, Univ. of Birmingham Medical School, UK.
2001-2004 Wellcome Trust UK Clinical Research Fellowship (International Travelling Prize), ANU
2007-2011 Sylvia and Charles Viertel Senior Research Fellowship (only 2 awarded per year in Australia)
2007 R.D. Wright Career Development Award Fellowship (NHMRC, declined)
2011-2017 Head, Department of Immunology and Infectious Disease, JCSMR, ANU
2011 Elizabeth Blackburn NHMRC Senior Research Fellowship: Highest ranked biomedical female fellow
2017 Elizabeth Blackburn NHMRC Principal Research Fellowship: Highest ranked biomedical female fellow

Other Recognition and Prizes

2007 Biogen-Idec III prize – Spain Dec 2007 – Prize to best research by Spanish scientist overseas.
2007 NSW Young Tall Poppy Science Award for extraordinary achievement in scientific research.
2008 Prime Minister Prize: Science Minister's Prize 2008 (Australian Life Scientist of the year).
2009 Gottschalk medal, Australian Academy of Science.
2012 Medal of Official of the Order of Queen Isabella the Catholic Awarded by the King of Spain.
2014 Inaugural CSL-Young Florey Medal.
2015 Fellow of the Australian Academy of Science (FAA).
2015 Bragg Member (Royal Institution of Australia).
2016 Rammaciotti Medal.

Translational impact

LOCAL

- Established the Centre for Pesonalised Immunology (CPI). CPI is a collaborative venture between patients, clinicians, bioinformaticians and scientists dedicated to personalised medicine, focused on the investigation of patients with autoimmune diseases and immune deficiencies that are undiagnosed, complex and difficult to treat. CPI investigators have sequenced ~ 2,000 genomes/exomes; described 12 new syndromes, identified and published 67 new human genome variations that lead to disease, and 28 novel cellular and biochemical mechanisms of human disease and invented 12 publicly accessible software applications for analysing human data and improving genomic diagnosis and discovery. This effort has improved outcomes for more than 300 patients through genomic diagnosis, ending the

diagnostic odyssey, and providing the basis for family planning, predictive testing and precision therapy, with several families with severe inflammatory conditions now being successfully treated. It has also helped meet the need for diagnostic clinical genomics diagnostics through the development of a publicly funded NATA-accredited clinical sequencing platform, Canberra Clinical Genomics.

- Trained 16 PhD students to completion, with several leading independent laboratories as well as 24 Honours students and 8 postdocs
- While Head of Immunology at ANU (2011-2018), built a department that attracted a large cohort of exceptional mid-career researchers and clinician scientists.

NATIONAL

- Helped coordinate, and led the Autoimmune Disease Theme of the Genetic Immunology (GI) rare disease flagship of the Australian Genome Health Alliance (AGHA), an NHMRC-funded health services research project to evaluate the place of genomics in clinical practice. This flagship constitutes the most substantial national clinical immunology network in Australia, with involvement from 12 centres in NSW, Qld, Vic, SA, WA, ACT.
- Direct engagement with media, and advocacy to politicians via Australian Academy of Science (AAS) fellowship and council membership.
- Patented two novel therapeutic targets for autoimmune disease in the last two years (2017902405; 2017903538 and has worked with CSL on one that is being taken through to commercialization.
- Provided national training in precision medicine and genomics through co-organisation of 2 CPI Schools of Personalised Immunology (Canberra 2015, Monash 2016).

INTERNATIONAL

- Her discovery of the roles of autoimmune disease (AD) regulators ICOS and IL-21 (Nature 2005; Nature 2007; JEM 2009) provided a rational for blockade of these molecules, currently being trialled in AD.
- Led the establishment of a CPI international network of clinical collaborators and a referral base extending to 13 countries on four continents: Europe, China (CACPI) and the Middle East with emerging collaborations in Africa, resulting in the recruitment of >1500 patients and multiple genomic diagnoses.
- Represents the CPI in the international Federation of Clinical Immunology Societies (FOCIS) contributing to accelerate multidisciplinary scientific and clinical innovation and education worldwide.

Research grants

•NHMRC Program Grant APP1113577 (2017-2021) CIH. •NHMRC Project Grant APP1125513 (2017-2021). CIA. •NHMRC Elizabeth Blackburn Senior Research Fellowship APP1117812 (2017-2021). •NHMRC, Centre for Research Excellence – Centre for Personalised Immunology (2014-2019). •NHMRC Targeted Research Call (2012-2014) CIC. •NHMRC Program Grant APP1016953 CID 2012-2016. •NHMRC Elizabeth Blackburn Senior Research Fellowship APP1021148. 2012-2016. •Human Frontiers Science Program RGP0033. CIC 2015-2017. •Vasculitis Foundation CIA 2014-2015. •P7/39 KU Leuven-Interuniversity IAP. 2013-2015.

Publications

> 100 publications. Cited ~ 10,000 times (>100 citation/item). Lead author in ~70%. H-index 45. Published in Nature (6x), Cell (2x), Nature Medicine (2x), Immunity (11x), Nature Immunology (8x), Nature Communications (4x), Journal of Experimental Medicine (6x). Recent examples:

Jiang S., …. and Vinuesa C.G. ***Nature Comm***. 2019 In press. First report of contribution of rare variants to human non-monogenic lupus and identification of pathogenic mechanism and potential therapy.

Papa I, …. Doglioni C* and Vinuesa CG*. ***Nature***. 2017. Identification of dopamine-mediated synaptic transmission to accelerate antibody responses, basis for patent 2017902405 to boost vaccination.

CARDILLO GRAY PARTNERS

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12 October 2018

Professor Carola Garcia de Vinuesa
Co-Director, Centre for Personalised Immunology
NHMRC Centre of Research Excellence
ANU College of Health and Medicine
Peter Baume Building
The Australian National University
42 Linnaeus Way
CANBERRA ACT 2601

Via email only: carola.vinuesa@anu.edu.au

Dear Professor Garcia de Vinuesa,

Re: Kathleen Folbigg

We represent Kathleen Folbigg in the upcoming Inquiry into her convictions for the deaths of her four children.

Procedural

We understand that you have agreed to being engaged by us for the purpose of conducting DNA testing for the four Folbigg children, as well as the mother, Kathleen Folbigg (and potentially the father, Craig Folbigg), and preparation of a report as to the findings.

We therefore request that you perform “genetic autopsies” of the children by Genomic testing via Whole Exome Sequencing, and additional genomic tests such as CGH arrays, karyotyping, and WGS as required. We expect that any consultation or collaboration with other experts that you require will be undertaken at your discretion, on the understanding that those experts may also be called to give evidence at the Inquiry if their opinion is expressed in the report.

A request has been sent to the Coroner’s Court regarding access to any remaining tissue samples of the Folbigg children in order that you can obtain whatever you require, and we will inform you as soon as those samples become available. The request has been made to allow you, or your delegate, to have access but we can provide you with a letter of authority if

required.

We ask that you prepare a report detailing your findings, including an explanation of the tests you conducted. We also ask that you provide an opinion as to whether the children's deaths can be attributed to a genetic cause, or causes, based on the tests you conducted. If you are able to determine that the children's deaths can be attributed to a genetic cause, we ask that you explain why, and, if possible, innumerate the strength of your opinion. If you find there is no clear genetic cause of death, we ask that you please explain what that means. In addition, could you please also articulate any limitations in your testing and the likelihood any errors occurred.

To assist in the preparation of your report, we attach the following documents:

1. Report and opinion in the case of Kathleen Folbigg by Professor Stephen Cordner
2. Autopsy report Caleb Folbigg, Patrick Folbigg, Sarah Folbigg, Laura Folbigg
3. Patrick blue book
4. Patrick EEG report 18/10/1990
5. Patrick EEG report 05/11/1990
6. Sarah Folbigg medical notes
7. Kathleen Folbigg antenatal records 1989
8. Report by Professor Ian Hutchinson
9. Medical post mortem documents for all 4 babies
10. Dr Bridget Wilcken screening request and result letter

Please ensure that annex to your report the following:

1. Relevant qualifications; (please annex a copy of your CV)
2. Any literature referenced and other significant material used in arriving at the opinion;
3. Any opinions or research relied upon;
4. Instructions, written or oral, which have affected the scope of the report.

Should you require further assistance or information, please feel free to contact us.

Yours faithfully,
CARDILLO GRAY PARTNERS

Stuart Gray
Partner

Inquiry into the convictions of Kathleen Megan Folbigg

7 February 2019

Professor Carola Garcia Vinuesa
Director, NHMRC Centre of Research Excellence
Centre for Personalised Immunology
John Curtin School of Medical Research
Australian National University
131 Garman Road
ACTON ACT 2601

By email: carola.vinuesa@anu.edu.au
cc: stuart.gray@cardillograypartners.com.au

Dear Professor Vinuesa

Preparation of expert report

We note that you have been instructed by Stuart Gray of Cardillo Gray Partners, solicitor for Kathleen Folbigg, to prepare a report for the Inquiry.

As discussed at Monday's meeting, to ensure consistency in the form of reports prepared for the Inquiry and that appropriate procedures are adhered to, please comply with the following in the preparation of your report.

Your report should only offer opinions to the extent those opinions are based upon your knowledge, training and fields of specialist expertise.

In preparing your report, please:

i. identify (and reference as appropriate) any facts and assumptions from materials upon which you rely;

ii. show how those facts and assumptions relate to your opinions;

iii. provide an explanation of your reasons for each of your opinions;

iv. define and explain any technical terms; and

v. if necessary, set out any qualification or reservations you have about the opinions expressed in your report (for instance, because of reservations you hold about a fact, or if further information is required, or for any other reason).

Your report should also clearly identify and reference any literature or other material on which you rely, and copies of such material should be annexed to your completed report, together with a copy of your curriculum vitae.

Expert code of conduct and curriculum vitae

At **Annexure A** to this letter I set out the Expert Witness Code of Conduct and ask that you read it carefully. In your report you should acknowledge that you have read the Code and agree to be bound by it. I suggest the following form of words be included in the body of the report:

> "I, Professor Carola Garcia de Vinuesa, acknowledge that for the purpose of Rule 31.24 of the Uniform Civil Procedure Rules 2005 that I have read the Expert Witness Code of Conduct in Schedule 7 to the said rules and agree to be bound by it."

Confidentiality

The Inquiry treats all information arising from the genetics data sequencing exercise, including raw data and reports about that data, as confidential and not for publication. A direction will be made shortly to this effect. Accordingly, please keep the genetics sequencing data, information resulting from the interpretation of that data and your report(s) confidential.

Please do not hesitate to contact Amber Richards, Senior Solicitor, on (02) 9258 0832 or amber.richards@cso.nsw.gov.au if you have any queries in relation to the above.

Kind regards

A.B. Richards

Amber Richards
Senior Solicitor
for Crown Solicitor

ANNEXURE A

Uniform Civil Procedure Rules 2005, Sch 7: Expert Witness Code of Conduct

1 Application of code

This code of conduct applies to any expert witness engaged or appointed:

(a) to provide an expert's report for use as evidence in proceedings or proposed proceedings, or

(b) to give opinion evidence in proceedings or proposed proceedings.

2 General duties to the Court

An expert witness is not an advocate for a party and has a paramount duty, overriding any duty to the party to the proceedings or other person retaining the expert witness, to assist the court impartially on matters relevant to the area of expertise of the witness.

3 Content of report

Every report prepared by an expert witness for use in court must clearly state the opinion or opinions of the expert and must state, specify or provide:

(a) the name and address of the expert, and

(b) an acknowledgement that the expert has read this code and agrees to be bound by it, and

(c) the qualifications of the expert to prepare the report, and

(d) the assumptions and material facts on which each opinion expressed in the report is based (a letter of instructions may be annexed), and

(e) the reasons for and any literature or other materials utilised in support of each such opinion, and

(f) (if applicable) that a particular question, issue or matter falls outside the expert's field of expertise, and

(g) any examinations, tests or other investigations on which the expert has relied, identifying the person who carried them out and that person's qualifications, and

(h) the extent to which any opinion which the expert has expressed involves the acceptance of another person's opinion, the identification of that other person and the opinion expressed by that other person, and

(i) a declaration that the expert has made all the inquiries which the expert believes are desirable and appropriate (save for any matters identified explicitly in the report), and that no matters of significance which the expert regards as relevant have, to the knowledge of the expert, been withheld from the court, and

(j) any qualification of an opinion expressed in the report without which the report is or may be incomplete or inaccurate, and

(k) whether any opinion expressed in the report is not a concluded opinion because of insufficient research or insufficient data or for any other reason, and

(l) where the report is lengthy or complex, a brief summary of the report at the beginning of the report.

4 Supplementary report following change of opinion

(1) Where an expert witness has provided to a party (or that party's legal representative) a report for use in court, and the expert thereafter changes his or her opinion on a material matter, the expert must forthwith provide to the party (or that party's legal representative) a supplementary report which must state, specify or provide the information referred to in clause 3 (a), (d), (e), (g), (h), (i), (j), (k) and (l), and if applicable, clause 3 (f).

(2) In any subsequent report (whether prepared in accordance with subclause (1) or not), the expert may refer to material contained in the earlier report without repeating it.

5 Duty to comply with the court's directions

If directed to do so by the court, an expert witness must:

(a) confer with any other expert witness, and

(b) provide the court with a joint report specifying (as the case requires) matters agreed and matters not agreed and the reasons for the experts not agreeing, and

(c) abide in a timely way by any direction of the court.

6 Conferences of experts

Each expert witness must:

(a) exercise his or her independent judgment in relation to every conference in which the expert participates pursuant to a direction of the court and in relation to each report thereafter provided, and must not act on any instruction or request to withhold or avoid agreement, and

(b) endeavour to reach agreement with the other expert witness (or witnesses) on any issue in dispute between them, or failing agreement, endeavour to identify and clarify the basis of disagreement on the issues which are in dispute.

MATTHEW COOK
MB BS (Hons) PhD FRACP FRCPA FFSc (RCPA)

Academic Qualifications
Bachelor of Medicine, Bachelor of Surgery (*Hons.*), University of Sydney (1986)
Doctor of Philosophy, University of Sydney, 1998
Fellow, Royal Australasian College of Physicians, 1994.
Fellow, Royal College of Pathologists of Australasia, 1994.

Current Appointments
Director of Immunology, The Canberra Hospital
Professor of Medicine, Australian National University, ACT, Australia
Director of Centre for Personalised Immunology (NHMRC Centre of Research Excellence)
Immunopathologist, ACT Pathology
Director, Canberra Clinical Genomics

Fellowships and Prizes
Medical Foundation Fellowship, 1993–97; Excellence in Teaching Award, University of Sydney, 2002; Sylvia and Charles Viertel Clinical Investigator 2001–2003. Elected Foundation Fellow of the Faculty of Science, RCPA.

Recent competitive grant funding (category 1, past 5 years)

2017-2021	NHMRC Program Grant APP1113577 ($19,924,985). Regulation of Haemopoietic and Immune Cells in Health and Disease CI
2012-2016	NHMRC Program grant APP1016953 ($15,718,075). Molecular and cellular basis of inflammatory and immunodeficiency diseases CI
2014-2018	Centre for Research Excellence APP1079648. ($2,500,000) Centre for Personalised Immunology CIB
2016-2020	Targeted Call for Research APP1113531 $25,000,000. Preparing Australia for Genomic Medicine
2016-2019	NHMRC Project grant APP1107464 $787 600. How does NF-kB2 regulate thymic selection to prevent organ-specific autoimmune disease? CIA
2013-2015	NHMRC Project grant APP1049760 $517 097.10. Genomic medicine for human immune deficiency. CIA
2017-2019	**Cancer Australia** Project grant APP1130330. $574,009.00. AUTO-CHECK study: Molecular determinants of autoimmunity and immune related adverse events in advanced cancer patients treated with immune checkpoint inhibitors. CIA

Peer recognition Invited to speak at >70 national and international meetings, including six as plenary speaker. Regularly invited to provide peer reviews: *J Immunol, J Exp Med, Nature Med*, *Immunity, MJA, Postgrad Med J, Rheumatology*, *Arthritis Rheum, PLoS One, Front Immunol*
Invited grant reviews for NHMRC, MS Society of Australia; Australian Rotary Health Fund, Institute for Genomic Medicine, Taiwan; Arthritis UK; Reumafonds, The Netherlands. Editorial Board, Clinical and Translational Immunology (Nature publishing group)

Professional leadership
a. National Health and Medical Research Council
Project grant review panel, 2009, 2011, 2012,
Fellowship panel, 2017-18
Translational Faculty Member, 2013–
Faculty Steering Group, 2013–

a. Royal Australasian College of Physicians
College Research Committee, 2013–
College Education committee, 2008–2012
Joint Specialist Advisory Committee, Immunology, 2007–2012 (Chairman, 2008–2012)

Curriculum Committee, Immunology, Royal Australasian College of Physicians, 2009–12
Coordinator of Advanced Training in Immunology, 2007–08

b. ACT Health and ANU
College of Medicine Biology and Environment Research Committee 2013–
Australian Medical School Executive 2014–
ACT Health Medical Advisory Council 2016–

Publication record
I have published more than 95 peer–reviewed articles with an average of 68 citations per article (1994–2018, inclusive). This work has been published in good quality journals, including *Nature* (n=1, IF 42.4), *Immunity* (n=4, IF 19.8), *J Exp Med* (n=12, IF 13.9), and *Blood* (n=5, IF 9.8).

Selected recent publications (past 5 years):
Altin JA, … **Cook MC***, Goodnow CC*. *equal. Ndfip1 mediates peripheral tolerance to self and exogenous antigen by inducing cell cycle exit in responding CD4+ T cells. *Proc Natl Acad Sci, USA* 111 (6), 2067-2074. 2014.

Ellyard JI, … **Cook MC***, Vinuesa CG*. *Equal. Whole exome sequencing in severe early-onset SLE identifies a pathogenic variant in TREX1. *Arthritis Rheumatol*. 66(12):3382-6. 2014.

Lee CE, … **Cook MC**. Autosomal dominant B cell deficiency with alopecia due to a mutation in *NFKB2* that results in non-processible p100. *Blood* 124(19):2964-72. 2014

Taupin DR, …, **Cook MC**. A deleterious *RNF43* germline mutation in a severely affected serrated polyposis kindred. *Hum Gen Var***2**, Article number: 15013. 2015. doi:10.1038/hgv.2015.13

Wu Z, … **Cook MC**. Heterogeneity of human neutrophil CD177 expression results from *CD177P1* pseudogene conversion. *PLOS Genetics.* 2016. 26;12(5):e1006067. doi: 10.1371/journal.pgen.1006067.

Alshekaili J, …**Cook MC.** STAT3 regulates cytotoxicity of human CD57+ CD4+ T cells in blood and lymphoid follicles. *Sci Rep*. 2018 Feb 23;8(1):3529. doi: 10.1038/s41598-018-21389-8.

Dorjbal B, … **Cook MC***, Snow AL* (*equal) Hypomorphic CARD11 mutations associated with diverse immunologic phenotypes with or without atopic disease. *J Allergy Clin Immunol. 2018 Aug 28. pii: S0091-6749(18)31201-6. doi: 10.1016/j.jaci.2018.08.013. [Epub ahead of print]*

Cardinez C, Miraghazadeh B, …**Cook MC**. Gain-of-function IKBKB mutation causes human combined immune deficiency. *J Exp Med*. 2018 Nov 5;215(11):2715-2724

da Silva EN, … **Cook MC**. Kidney disease impairs follicular helper T cell activation after vaccination. *PLOS One* 2018 Oct 10;13(10):e0204477

Jiang SH, Athanasopoulos V, …**Cook MC** and Vinuesa CG. Rare and low frequency variants in *BLK* and *BANK1* impair IRF5 repression and predispose to systemic autoimmunity. *Nature Commun*. In press.

Inquiry into the convictions of Kathleen Megan Folbigg

21 December 2018

Professor Matthew Cook
Director
Department of Immunology
Canberra Hospital
Yamba Drive
GARRAN ACT 2605

By email: matthew.cook@anu.edu.au

Dear Professor Cook

Letter of Engagement

Background

On 22 August 2018 the Governor of New South Wales directed that an inquiry be held into the convictions of Kathleen Megan Folbigg on 21 May 2003 for three counts of murder, one count of manslaughter and one count of maliciously inflicting grievous bodily harm in respect of her four children ("the Inquiry"). The Crown Solicitor is the Solicitor Assisting the Honourable Reginald Oliver Blanch AM QC ("the Judicial Officer") with the Inquiry.

The scope of the Inquiry includes consideration of expert medical evidence, including:

- any new research or advances in medical science relevant to the causes of death of each child and the cause of the apparent or acute life threatening event in respect of one child, Patrick.
- expert medical opinion as to the causes of death of each child and the cause of the apparent or acute life threatening event in respect of Patrick in light of any relevant new research or advances in medical science.
- any new research or literature concerning the incidence of reported deaths of three or more infants in the same family attributed to unidentified natural causes.
- any other related expert medical evidence.

Scope of engagement

As discussed you are engaged, as one of a team of experts, to interpret raw data produced as a result of genetic sequencing and testing arranged by the Inquiry in respect of the Folbigg family, and provide an expert report to the Inquiry regarding the presence of any

likely pathogenic genes, or pathogenic genetic variants in genes, that are known to be associated with sudden unexpected death in infancy.

As per our correspondence to you dated 20 December, we anticipate the data will be available by early February 2019.

We confirm the team of experts the Inquiry is engaging comprises:

- Dr Michael Buckley MBChB PhD FRCPA FHGSA FRCPath: senior staff specialist genetic pathologist employed by NSW Health Pathology and currently the President of the Human Genetics Society of Australasia

- Professor Edwin Kirk MBBS PhD FRACP FRCPA: senior staff clinical geneticist and genetic pathologist employed by NSW Health Pathology and Chief Examiner in Genetics for the Royal College of Pathologists of Australia

- Dr Alison Colley MBBS FRACP FRCPA: senior staff specialist clinical geneticist in specialist practice at Liverpool Hospital and Director of the Liverpool Genetics Service.

It is possible that you will be required to give oral evidence at the public hearings of the Inquiry. It is expected the hearing relevant to genetics will be held in March 2018. We will advise you as soon as the hearing dates have been listed.

Preparation of your report

The Inquiry would be assisted if you could interpret the results of the genetic testing and prepare a report which identifies and explains whether any of the results of that testing are relevant to the causes of death of each of the children.

Your report should only offer opinions to the extent those opinions are based upon your knowledge, training and fields of specialist expertise.

In preparing your report, please:

i. identify (and reference as appropriate) any facts and assumptions from materials upon which you rely;

ii. show how those facts and assumptions relate to your opinions;

iii. provide an explanation of your reasons for each of your opinions;

iv. define and explain any technical terms; and

v. if necessary, set out any qualification or reservations you have about the opinions expressed in your report (for instance, because of reservations you hold about a fact, or if further information is required, or for any other reason).

Documents with which you are briefed

As you know, in advance of the meeting of geneticists on 10 December, we provided you with a set of briefing documents. For your reference, the index to this set is set out below in **Annexure A**. We will provide you with any other necessary documents as they come to hand.

Expert code of conduct and curriculum vitae

At **Annexure B** to this letter I set out the Expert Witness Code of Conduct and ask that you read it carefully. In your report you should acknowledge that you have read the Code and agree to be bound by it. I suggest the following form of words be included in the body of the report:

> "I, Professor Matthew Cook, acknowledge that for the purpose of Rule 31.24 of the Uniform Civil Procedure Rules 2005 that I have read the Expert Witness Code of Conduct in Schedule 7 to the said rules and agree to be bound by it."

I also request that you please attach a copy of your curriculum vitae to your report.

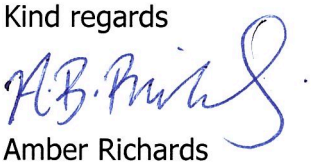
Confidentiality

Please ensure you keep your engagement, the documents with which you are briefed, and your report **confidential**.

Conclusion

Please do not hesitate to contact Amber Richards, Senior Solicitor, on (02) 9258 0832 or amber.richards@cso.nsw.gov.au if you have any queries or require anything further to assist in the preparation of your report.

Kind regards



Amber Richards
Senior Solicitor
for Crown Solicitor

Encl. (1)

ANNEXURE A

Index to briefing material

Tab	Document	Date	Source
1.	Medical testing of Patrick Folbigg	13 February 1991	ODPP Volume 1 of 7, Tab 29
2.	Letter to Doctor Wilcken regarding newborn blood sample	11 October 1999	ODPP SYD02582197, Tab 168
3.	Genetics report regarding death of Caleb Folbigg	13 January 2000	SC054200, Tab 211
4.	Genetics report regarding death of Patrick Folbigg	13 January 2000	SC054200, Tab 212
5.	Genetics report regarding death of Sarah Folbigg	13 January 2000	SC054200, Tab 213
6.	Genetics report re death of Laura Folbigg	13 January 2000	SC054200, Tab 214
7.	Expert Certificate / Statement of Doctor Bridget Wilcken and exhibits 7a – 7c	14 January 2000	ODPP SYD02583893, Tab 98
7a.	Letter from Doctor Alison Colley to Doctor Bridget Wilcken regarding Caleb and Patrick Folbigg	4 December 1991	ODPP SYD02583893, Tab 100
7b.	Letter from Doctor Bridget Wilcken to Doctor Alison Colley regarding Caleb and Patrick Folbigg	10 December 1991	ODPP SYD02583893, Tab 101
7c.	NSW Newborn Screening Programme Report regarding Caleb, Patrick, Sarah and Laura Folbigg	13 January 2000	ODPP SYD02583893, Tabs 103-106
8.	Report of Professor Peter Berry	November 2000	ODPP SYD2575735, Tab 25
9.	Letter from Doctor Alison Colley to Doctor Bridget Wilcken	27 February 1992	ODPP SYD02583893, Tab 102
10.	Letter from Professor David Isaacs re testing for serum levels of IgG of Folbigg children	3 March 2003	ODPP SYD02584119, Tab 109
11.	Facsimile from Dr J Vivian Wells re IgG levels	5 March 2003	ODPP SYD02584119, Tab 120
12.	Medical Testing for IgG deficiency, prolonged QT and "Druckers" Gene	7 March 2003	SC54199, Tab 27
13.	Letter from Doctor Allan Cala re IL-10 Gene theory	19 March 2003	ODPP SYD02582197, Tab 13
14.	Letter from Doctor John Christodoulou to J Culver regarding genetic causes of some cases of SIDS	18 February 2003	ODPP SYD02584119, Tab 89
15.	Summary of present situation re medical investigations prepared by Peter Krisenthal, Legal Aid	27 February 2003	ODPP SYD02584119, Tab 101

Tab	Document	Date	Source
16.	Letter from Professor John Hilton to ODPP regarding genetic testing of Folbigg Children	27 February 2003	DPP SYD02584119, Tab 100
17.	Transcript of evidence of Doctor Bridget Wilcken at trial (pages 817-823)	16 April 2003	Initial Transcripts bundle Volume 3, Tab 11 (provided with Preliminary Bundle)
18.	Supplementary Report of Professor Peter Berry regarding Doctor Drucker's work on IL-10 gene polymorphism theory	29 April 2003	ODPP SYD02584119, Tab 188
19.	Final Report of Professor Cecelia Blackwell	8 May 2014	Professor Cecelia Blackwell
20.	Article, "Exploring the risk factors for sudden infant deaths and their role in inflammatory responses to infection" (Frontiers in Immunology, Volume 6, Article 44) by Caroline Blackwell, Sophia Moscovis, Sharron Hall, Christine Burns and Rodney J Scott	March 2015	N/A
21.	Chapters from Book, *SIDS - Sudden infant and early childhood death: The past, the present and the future* edited by Jhodie R Duncan and Roger W Byard (University Adelaide Press 2018):	N/A	N/A
21a.	Chapter 2 – Sudden Infant Death Syndrome: An Overview by Jhodie R Duncan and Roger W Byard	N/A	N/A
21b.	Chapter 14 – Future Directions in Sudden Unexpected Death in Infancy Research by Heather E Jeffery	N/A	N/A
21c.	Chapter 30 – Cytokines, Infection, and Immunity by Siri Hauge Opdal	N/A	N/A
21d.	Chapter 31 – The Genetics of Sudden Infant Death Syndrome by Catherine A Brownstein, Annapurna Poduri, Richard D Goldstein and Ingrid A Holm	N/A	N/A
22	Specimen slides relating to Caleb, Sarah, Laura and Patrick Folbigg – Working Document	15 November 2018	N/A
23	NSW Forensic & Analytical Science Service - Histology Request Forms	N/A	N/A

ANNEXURE B

Uniform Civil Procedure Rules 2005, Sch 7: Expert Witness Code of Conduct

1 Application of code

This code of conduct applies to any expert witness engaged or appointed:

(a) to provide an expert's report for use as evidence in proceedings or proposed proceedings, or

(b) to give opinion evidence in proceedings or proposed proceedings.

2 General duties to the Court

An expert witness is not an advocate for a party and has a paramount duty, overriding any duty to the party to the proceedings or other person retaining the expert witness, to assist the court impartially on matters relevant to the area of expertise of the witness.

3 Content of report

Every report prepared by an expert witness for use in court must clearly state the opinion or opinions of the expert and must state, specify or provide:

(a) the name and address of the expert, and

(b) an acknowledgement that the expert has read this code and agrees to be bound by it, and

(c) the qualifications of the expert to prepare the report, and

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(1) Where an expert witness has provided to a party (or that party's legal representative) a report for use in court, and the expert thereafter changes his or her opinion on a material matter, the expert must forthwith provide to the party (or that party's legal representative) a supplementary report which must state, specify or provide the information referred to in clause 3 (a), (d), (e), (g), (h), (i), (j), (k) and (l), and if applicable, clause 3 (f).

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(a) confer with any other expert witness, and

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6 Conferences of experts

Each expert witness must:

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