

EXHIBIT BW

11 July 2019

Response to Supplementary Report by Prof. Kirk, Dr Buckley and Prof. Skinner dated 5 July 2019

1. Introduction

- 1.1. In their supplementary report, the Sydney team reanalyses the *CALM2* p.G114R variant found in Kathleen, Sarah and Laura Folbigg in light of Professor Schwartz's letter dated 20 June 2019 and his recent paper (Crotti L *et al.*, 2019). In addition, the Sydney team take into account the rarity of variants within the same protein domain as the *CALM2* G114R variant based on a recent publication that describes a new tool for testing this well-established principle (a principle we had applied in our original analysis). After applying the American College of Medical Genetics (ACMG) guidelines, and based on available facts, the Sydney team has revised their classification of the *CALM2* G114R variant as likely pathogenic. Based on opinion and speculation, however, they have classified the variant as non-pathogenic. We recommend that any conclusions should be based on available facts together with an acknowledgement of the current limits of our knowledge. Furthermore, we suggest that reaching a conclusion based on speculation rather than acknowledgement of these available facts and knowledge gaps is *fundamentally flawed*. Very recent information on the precise *CALM2* genetic variant in question exemplifies why we should be careful not to discount possibilities until the facts are in.
- 1.2. Here, we outline the facts as they stand, and the principles which challenge speculation by the Sydney team. The following addresses each of the points the Sydney team raise as a basis for rejecting the ACMG guidelines and resiling from a finding that the G114R variant should be classified as likely pathogenic.

2. Response to Sydney team claim 1: unlikely clinical scenario

- 2.1. The Sydney team assert that the *CALM2* G114R variant is not pathogenic due to the unlikely clinical scenario of different genetic causes for the deaths of the four children and their speculation on expectations of how the G114R variant might behave.
- 2.2. Sydney team: *"... there was no reason prior to the genetic results to consider Sarah and Laura as likely to have had a different genetic cause of death from Caleb and Patrick..."* Segregation has been called into question but as we have emphasised previously including in the preamble to our initial genetic report, there is uncertainty about the phenotypes that require explanation. Contrary to the Sydney team, we do not consider death a phenotype. Instead, our starting point is that the phenotype that caused death in each child remains ambiguous, a conclusion supported by the relevant and inconsistent clinical and pathological analyses of the Folbigg children. There are no facts to refute the possibility that there was more than one cause of death. We are not aware of any way in which the odds of this possibility could be estimated. Segregation should therefore be considered according to more than one scenario.
- 2.3. Sydney team: *"Any discussion of the CALM2 variant as a possible cause of death for Sarah and Laura must necessarily assume that there are at least two different causes of death for*

the children – one for Sarah and Laura, and one or more for Caleb and Patrick". There are numerous examples where two different monogenic conditions causing similar (or different) clinical presentations manifest in a single family and even in a single individual, leading to manifestation of two different phenotypes and/or modified (blended or aggravated) clinical manifestations. Recent literature indicates that as many as 5% (or 1 in 20) cases referred for whole-exome testing are found to have 2 monogenic conditions (Posey et al., 2018). In addition, members of the same family have been shown to develop the same condition due to pathogenic variants in different genes, for example, breast cancer in different members of the same family due to mutations in either BRCA1 or BRCA2 (Leegete B et al., 2005; Lavie et al., 2011; Heidemann S et al., 2012; Nomizu T et al., 2015). We are not aware of any facts to refute that the two female Folbigg children died as a result of the CALM2 G114R variant, while the two male children died from different causes, whether genetic or otherwise.

- 2.4. The two male Folbigg children also had other mutations in genes known to cause Brugada syndrome when mutated. For example, Patrick had a mutation in SCN1A (F296V) and Caleb (as well as the female Folbigg children) had a mutation in KCNB2 (A11T), in a residue adjacent to two other residues found to cause Brugada Sd: p.R12Q, and p.L13F (Portero V et al., 2016).
- 2.5. Sydney team's claim: *"The variant is of a type that (if pathogenic) would not usually be expected to cause death at such a young age"*. Making assumptions rather than acknowledging current uncertainty is risky. The Sydney team concluded initially (and stated at the hearing / recorded in the transcript) that Laura Folbigg was 'too old' to fit the criteria for SIDS (death under 12 months of age) or deaths caused by all known CALM gene family mutations. Now the Sydney team states that Laura is 'too young' to fit the CALM G114 or CALM-CPVT-related death scenario (death outcome above age 4 years). By contrast, we recommend acknowledgement of the **well-established and accepted basic principles in formal genetics of variable clinical expressivity** (variable age of onset and variable severity of disease caused by the same genetic mutation or different mutations in the same gene) **and blended/masked/mistaken phenotypes** (leading to discovery of new conditions). Precise amino acid substitution, rather than exclusively the location within the protein, can influence the type of protein damage and disease expression (including age of onset). Variable expressivity and variable age of onset depending on the precise amino acid substitution is very much the case for calmodulin mutations as described in the recent paper by Prof. Schwartz (Crotti 2019, Eur H J) and in the editorial by Prof. Michael Overgaard (Nyegaard, 2019, Eur H J), or in any of a number of recent reviews on calmodulin mutations.

3. Response to Sydney team claim 2: healthy carriers

- 3.1. The Sydney team's assertion that a CALM2 gene variant cannot be disease-causing if present in a phenotypically healthy carrier is incorrect. They state: *"These [cardiac examination] results and the fact that she is alive and has never had a cardiac arrest is strongly against this hypothesis; it would be very unusual to have a disease causing variant responsible for deaths at such young ages also present in a healthy person in her 50s"*. They also state: *"The variant is of a type that (if pathogenic) would almost always*

be expected to have arisen de novo or have been inherited from a parent with mosaicism for the variant". These statements are incorrect.

3.2. **A 'healthy' related person can be the carrier of a mutation that can be lethal in another related person.** This genetic phenomenon is called "incomplete penetrance" and there are several explanations widely accepted by modern genetics (and remarkably still not captured by the ACMG guidelines), which cover such a situation. Reasons for this include:

3.3. Mosaicism

- a) Mosaicism describes the phenomenon where a person with a deleterious variant appears phenotypically healthy as they do not carry the mutation in one or several tissues. This phenomenon arises due to a *de novo* mutation occurring during cell division of a developing embryo as opposed to the mutation occurring in either parental sperm or egg.
- b) It is crucial to note that one previously documented healthy carrier of the *CALM3* p.G114W mutation (as described by Crotti *et al.*, 2019) was a mosaic. In other words, the pathogenic variant can be present in 50% of the DNA molecules in some tissues (for example blood and/or saliva), but absent from other tissues (such as the heart).
- c) The Sydney team states: "*based on the available genetic data [40:42 split between the normal and the variant base pair], mosaicism is very unlikely*". This is incorrect. Blood or buccal swab variant/reference allele DNA ratios would only inform mosaicism in hematopoietic and buccal epithelial/mucosal cells but not in cells from the heart. Invasive cardiac biopsy would be the only way to exclude relevant mosaicism that could protect Kathleen Folbigg from heart disease. Such invasive testing may be ethically unjustifiable in the context of the possibility that there is another known/testable or unknown/untestable explanation for the observed reduced penetrance in Kathleen Folbigg.

3.4. Digenic causes or gene modifiers

- a) This refers to the situation where two separate variants which interact, or act in related pathways, are required for a more pronounced phenotype. An example in LQTS, is the relatively common *KCNH2* K897T mutation (30% carrier frequency) which modifies expressivity of latent *LQT2* mutations resulting in a more severe and lethal phenotype (Crotti L *et al.*, 2019).
- b) There were several VUS in the children that were borderline in their classification of pathogenicity and may be proven to be pathogenic in the future that could interact with the *CALM2* variant to cause disease (i.e. a digenic cause of SUD). These can act either as "second hits" or modifier genes. For example, the *KCNAB2* Ala11Thre variant present in Caleb, Sarah and Laura was presumably inherited from the father (or might have occurred *de novo* in the children due to parental gonadal mosaicism) and if pathogenic, could contribute to exacerbate the phenotype in the children.

3.5. Environmental trigger

- a) This can include a requirement of a specific environmental trigger during a particular developmental stage in order for a lethal outcome, an example being an infection in early childhood. Indeed, fever has shown to be a trigger of cardiac arrhythmias in

childhood in the context of *SCN5A* and *KCNH2* mutations. Amino acid residues that change protein stability are likely to make the protein more unstable with higher temperature (i.e. fever), and this can occur in any protein, and may be the case for *CALM2* G114R variant. In other words, the reason why Kathleen Folbigg has not experienced the “major phenotype” (sudden death in early childhood) is due to a lack of environmental trigger in the critical developmental stage.

- 3.6. With respect to the incomplete penetrance and seemingly healthy individuals in the context of LQTS, in 1980 Professor Schwartz first posited that **there could be patients with LQTS and completely normal ECG**. This hypothesis was anecdotally confirmed shortly thereafter (see Kulbertus & Wellen, 1980). We have enclosed Professor Schwartz’s chapter from that book with the relevant page highlighted (p 376). That page describes a family with eleven children of whom five suffered sudden (unexplained) death. ECG examination of the six remaining children revealed 5 had a prolonged QT intervals while the sixth child was normal. One week after the examination the sibling with the normal ECG died suddenly in his bed, as occurred with the Folbigg children. In 1999 Priori *et al.* confirmed Professor Schwartz’ hypothesis with genetic evidence (Priori *S et al.*, 1990). **Thus, it is now accepted that there are patients with LQTS and completely normal ECG that appear healthy but are at risk of sudden cardiac death.**

- 3.7. With respect to Sydney team’s claim: “*The variant is of a type that (if pathogenic) would almost always be expected to have arisen de novo or have been inherited from a parent with mosaicism for the variant*”. This is incorrect. Marsman *et al.* (2014) report a family with idiopathic ventricular fibrillation (IVF) caused by *CALM1* (p.F90L) manifesting in childhood and adolescence (we have enclosed a copy of this paper). IVF was also reported as the cause of deaths of the two children with the G114W variant inherited from a healthy mother (mosaic) (Crotti *et al.*, 2019). In Marsman *et al.*’s paper the *CALM1* (p.F90L) variant was also carried by a seemingly healthy mother having five children with the variant, 2 of which died from SUD, two with syncopes and cardiac arrests, while the fifth remained asymptomatic. Moreover, this variant is found in an interdomain linker region of Calmodulin rather than in a calcium binding region, as is the variant identified in Kathleen Folbigg and her children. **In summary, the facts reveal that there are now increasing reports of healthy carriers of lethal CALM variants that cause IVF/SUD.**

4. Response to Sydney team claim 3: Kathleen Folbigg’s cardiac evaluation

- 4.1. The Sydney team assert that Kathleen Folbigg’s ECG results indicate she is not at risk for LQTS, CPVT or sudden death. This is incorrect.
- 4.2. *Notwithstanding the above discussion about incomplete penetrance, the conclusion that Kathleen Folbigg is unaffected by the variant is still premature.*
- 4.3. As reported by Dr Raju, Kathleen Folbigg has had a long-standing history of recurrent transient loss of consciousness/syncopes that started in childhood. She had multiple faints in childhood, often during intercurrent illnesses, and as a teenager she recalled multiple episodes of sudden post-exertional syncope, some of them witnessed (one while swimming requiring her to be dragged out of the pool). There have also been several syncope episodes recorded in her prison health records made available to the inquiry, at least one witnessed and one causing a n injury to the head and bleeding requiring medical attention. The cause of these syncopal episodes remains unknown. There is **evidence that**

LQTS can manifest with both stress-related *and neurogenic* syncopes, as we cited in the Inquiry (Liu FJ *et al.*, 2011; Nordkamp LRO *et al.*, 2011; Toft E *et al.*, 2003,).

- 4.4. Professor Schwartz has stated that ***normal QT intervals can be found in people at risk of sudden death due to LQTS*** (this is reflected in the Schwartz diagnostic criteria for LQTS, and incorporated in the CSANZ LQTS Guidelines, authored/reviewed by Prof Skinner). The *CALM2* variant identified in Kathleen and her children is likely to cause a cardiac condition amidst the CPVT/LQTS/IVF spectrum. This opinion is shared by a calmodulin expert and biochemist Professor Michael Toft Overgaard. Professor Schwartz also considers that while CPVT is more likely, LQTS is also possible.
- 4.5. *We conclude that there remain uncertainties regarding the clinical phenotype of Kathleen Folbigg. In addition, even if she lacks clinical manifestations there are precedents that mean this cannot exclude pathogenicity of the variant in her offspring.*

5. Response to Sydney team claim 4: death during sleep is an unlikely clinical scenario for CALM variants

- 5.1. The Sydney team assert that the clinical presentation (death during sleep) of the Folbigg children is discordant with *CALM2* associated deaths (mostly while playing). They state *"To our knowledge, variants in calmodulin have still not been reported as a cause of sudden infant death syndrome, being death of an infant during sleep"*. This is not correct.
- 5.2. **The facts recorded in the Registry of Calmodulinopathies (Crotti et al. Eur Heart J 2019) are that there are five cases of sudden death/cardiac arrest while asleep caused by *CALM1/2/3* mutations.**
- 5.3. Variable genetic expressivity is well accepted in genetics, and we are already seeing a spectrum of clinical presentations caused by *CALM* variants. The actual mutation can be the cause of different presentations. For example, if the G114R variant is temperature-sensitive and changes protein stability, a fever may trigger the protein malfunction. There was evidence of infection in the Folbigg female children (lungs, myocarditis).

6. Summary

- 6.1. The Sydney team resiled from their classification of the *CALM2* G114R variant as likely pathogenic according to the ACMG guidelines based on a series of assertions relating to the clinical presentation of Kathleen Folbigg and her children.
- 6.2. The Sydney team's primary reason for departing from the ACMG guidelines appears to be because they believe Ms Folbigg is "healthy" and incorrect assumptions/knowledge about *CALM*-induced deaths. There is insufficient evidence that Ms Folbigg is unaffected by the G114R variant at this stage. Regardless, a finding that she is "healthy" is not a basis for overriding the ACMG criteria which support "likely pathogenic" interpretation of this variant.
- 6.3. The G114 codon variant of *CALM2* has now been identified in another kindred with sudden cardiac death.

- 6.4. In the absence of conclusive phenotypic information about the children, there remains no factual basis for dismissing the significance of the *CALM2* variant based on segregation analysis.
- 6.5. The Sydney team concludes that for the G114R variant to be pathogenic requires an “exceptional clinical scenario”. Although we have detailed why their clinical claims are largely incorrect, there is still general agreement that we are dealing with an exceptional clinical scenario. **Rare genetic variants are by definition responsible for exceptional clinical scenarios. We regularly deal with, and publish papers on, exceptional clinical scenarios.** The family reported in Crotti *et al.* 2019 with the G114W variant, which involved the sudden unexpected deaths of two children to a healthy mother, was an exceptional clinical scenario. We have always posited that this case should be treated as an exceptional clinical scenario and now that the Sydney team have agreed that the variant should be classified as “likely pathogenic” according to the ACMG guidelines, they reject the finding on the basis that this would involve accepting that this case presents an exceptional clinical scenario.
- 6.6. **Whether the clinical scenario is “exceptional” or not, has no bearing on the interpretation of the pathogenicity of the variant.** Even if this variant had not met the criteria to be classified as “likely pathogenic” per the ACMG criteria, we would maintain our position that applying the conservative and rigid ACMG criteria on the research end of the clinical/diagnostic genetics spectrum is not appropriate. This is also the view of the creators of the ACMG criteria, as outlined in the published ACMG guidelines (Richards *et al.*, 2015). Notwithstanding the stringency of the ACMG criteria the G114R variant has now been classified by both teams as likely pathogenic when adhering to the criteria.
- 6.7. **In conclusion, based on the available facts we cannot reasonably exclude, and we think it is likely, that the two female Folbigg children died as a result of the *CALM2* G114R variant, while the two male children died from different causes that could also be genetic.**

7. Review and Endorsement

- 7.1. This letter was drafted by Professor Carola Vinuesa and Dr Todor Arsov and reviewed, added to, and endorsed by Professors Peter Schwartz, Matthew Cook, and Michael Toft Overgaard.
- 7.2. In reviewing and endorsing this response, Professors Schwartz and Toft Overgaard were provided with the following exhibits in addition to the Supplementary Report by the Sydney team (dated 5 July 2019):
 - a) **AH** – ECG of Kathleen Folbigg dated 17 May 2011
 - b) **AE** – Pedigree of Kathleen Folbigg (family) dated 8 October 2018
 - c) **AF** – Joint report of Canberra team dated 29 March 2019
 - d) **Z** – Joint report of Sydney genetics team dated 29 March 2019
 - e) **Y** – Expert report of Professor Jon Skinner dated 31 March 2019
 - f) **AX** – Written response to joint expert report of Vinuesa and Cook dated 9 April 2019
 - g) **AY** – Written reply to response of Kirk and Buckley dated 12 April 2019
 - h) **BL** – Letter from Dr Hariharan Raju dated 18 April 2019
 - i) **BK** – Letter from Prof. Jonathan Skinner dated 30 April 2019

References

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CV for Michael Toft Overgaard – short version

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Education:

2000 PhD, Molecular Biology, Faculty of Science, University of Aarhus (AU),

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2018 – Head of Department, Department of Chemistry and Bioscience, Aalborg University (AAU)

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2009 – 14 Associate Professor in Protein Science, Dept. of Chemistry and Bioscience, AAU

2006 – 08 Associate Professor, Dept. of Molecular Biology, AU

2003 – 05 Postdoc, Dept. Structural Biology, Stanford University, USA

2000 – 03 Postdoc, Dept. Molecular Biology, AU

Scientific Profile: Broad methodological expertise, in biochemistry, physical biochemistry (including structural biology, mass spectrometry), protein chemistry, and molecular biology. Main focus areas are:

- intracellular calcium signalling and homeostasis – role of calcium binding proteins
- proteolytic enzyme discovery, characterization, substrate specificity and inhibition
- antimicrobial peptides

Board and Committee Memberships:

2019 – Board member, Danish Protein Innovation

2019 – Steering Committee, Health-Innovation-HUB @ AAU

2019 – Board member, Thisted High School

2013 – 2016 Co-founder, CSO and member of the Board for CamAgon ApS

2012 – Member of the Department Committee, Chemistry and Biotechnology, AAU

2011 Chairman, the Novo Nordisk - Center for Protein Research, PST-facility review panel

2010 – 2019 Chairman of the Chairmanship for External Biology Examiners in Denmark

2000 – Co-founder for COMO Biotech ApS (Chairman of the Board), and COMO Holding ApS (CEO).

Project Management and Supervision: Head of an active research group (currently 3 postdocs and 1 PhD student). Managing projects funded by FNU, the Lundbeck Foundation, the Novo Nordisk Foundation, the Innovation Fund Denmark (WP leader).

Patents: Co-inventor on 5 granted patents (U.S. Patents 9005949, 7115382 and 7083940, Japanese Patent 4460829, European Patent EP1169058) and 4 published patent applications.

Publications: 86 original peer reviewed research papers (7 first, 13 senior authorships), 1 book chapter. H-index: 38 (Web of Science), more than 4900 citations generated (June 2019).

Five most recent publications:

- 1) Nyegaard M, Overgaard MT. The International Calmodulinopathy Registry: recording the diverse phenotypic spectrum of un-CALM hearts. **Eur Heart J**. 2019
- 2) Søndergaard MT, Liu Y, Brohus M, Guo W, Nani A, Carvajal C, Fill M, Overgaard MT, Chen SRW. Diminished inhibition and facilitated activation of RyR2-mediated Ca²⁺ release is a common defect of arrhythmogenic calmodulin mutations. **FEBS J**. 2019
- 3) Sorensen AB, Madsen JJ, Frimurer TM, Overgaard MT, Gandhi PS, Persson E, Olsen OH. Allosteric in Coagulation Factor VIIa Revealed by Ensemble Refinement of Crystallographic Structures. **Biophys J**. 2019
- 4) Brohus M, Søndergaard MT, Wayne Chen SR, van Petegem F, Overgaard MT. Ca²⁺-dependent calmodulin binding to cardiac ryanodine receptor (RyR2) calmodulin-binding domains. **Biochem J**. 2019
- 5) Jensen HH, Brohus M, Nyegaard M, Overgaard MT. Human Calmodulin Mutations. *Front Mol Neurosci*. 2018