EXHIBIT AX

9th April, 2019

Re: the report entitled "Initial Report into the Genetic Sequencing of the Folbigg Family", dated 29th March 2019, by Professor Vinuesa and Professor Cook.

Response by Dr Michael Buckley and Prof Edwin Kirk

We set out our responses to the variants referred to in the report by Professor Vinuesa and Professor Cook below. We have referred to paragraph numbering as it appears in that report

Specific genes and variants:

***DMPK* (document paragraph 7.2 and following)**

The *DMPK* triplet expansion results in formation of a toxic RNA; the known phenotypes associated with *DMPK* are not caused by loss of function or missense variants in *DMPK* itself.

7.3.1 Sabovic et al 2003 [PMID 14678805] state that the evidence that CTG repeat expansion [is a] potentially important predictor of cardiac involvement and sudden death in patients with DM is inconclusive. The authors conclude that CTG expansion has no role in predicting either conduction abnormalities or sudden death in their patients. They do not make claims that other variants in *DMPK* are associated with sudden death.

7.3.1 Wahbi et al (2013) [PMID 24140416] investigated *SCN5A* splicing in individuals with ECG evidence of Brugada syndrome who also had myotonic dystrophy. They do not make claims that other variants in *DMPK* are associated with altered splicing of *SCN5A*. Where available, no member of the Folbigg family has a Brugada pattern on ECG.

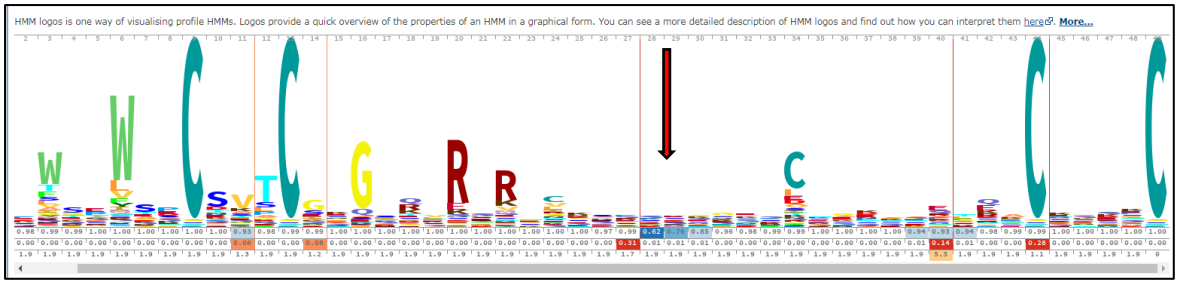
7.3.2 Pambrun et al (2014) [PMID 24768612] describe a single family with putative digenic interaction between a paternally inherited *DMPK* CTG expansion and maternally inherited *SCN5A* variant. No member of the Folbigg family has an *SCN5A* variant. The publication does not address missense changes in *DMPK*. A single family report of a putative digenic interaction in the absence of comprehensive genome scan for other contributory sequence and copy number variants is essentially meaningless. Even if this interaction were confirmed, it would not be relevant to the identification of a missense variant in *DMPK.*

7.4 The homozygous mouse mutants are irrelevant, as these are homozygous loss of function variants compared with the heterozygous missense change which is predicted to be benign.

***ADAMTS6* (document paragraph 7.5 and following)**

There is no known human Mendelian disease phenotype associated with variants in this gene.

7.5.1 the variant is in the TSP type 1 domain. The Glycine at this position is not conserved among paralogous TSP-1 domains - see below HMM logo for TSP-1 domains – amino acid of interest arrowed). Glycine 592 does not form a disulphide bond, as glycine does not contain a thiol group and thus is incapable of participating in disulphide bond formation (cysteine is the only amino acid that has this capacity).



7.5.2 Prins et al. (2018) [PMID: 30012220] This is a genome-wide association study conducted in adult individuals who could only be included if they did not have any known disorder of cardiac conduction. It found that variation in QRS duration of just *0.72 milliseconds* could be accounted for by variants affecting this gene.

The variant is present in 1 in 6,426 non-Finnish Europeans in gnomAD; it is too common for a disease causing variant in relation to a severe and very rare phenotype.

***SLC12A9* (document paragraph 7.6 and following)**

There is no evidence linking variants in this gene to human Mendelian disease.

Eijgelsheim et al (2010) [PMID 20639392]. The paper referred to is a genome-wide association study, this time a study of resting heart rate in adults. The phenotype in that study is not relevant to the phenotype of SUDI.

There are 4 heterozygous alleles of this variant in the population database gnomAD.

***TAB1* (document paragraph 7.7 and following)**

There is no evidence linking variants in this gene to human Mendelian disease.

This variant is present in 37 alleles in the population database gnomAD, highest frequency being in South Asians (1 in 1,531 individuals carry the variant, which exceeds the frequency of SUDI, estimated at 1:3300 in Australia).

***KCNQ1 deletion* (document paragraph 8.6.3 and following)**

8.6.3 The deletion variant provided is located deep within intron 15 of *KCNQ1* and is essentially identical to the common Chr11(GRCh37):g.2814641_2814711del variant in KCNQ1 which is present in the normal population database gnomAD at a heterozygote frequency of 23.14% in non-Finnish Europeans, and homozygote frequency of 5%.

Following the ACMG criteria this variant is classified Benign.

***SEMA3A* (document paragraph 8.6.4 and following)**

The identified variant is a duplication of the segment Chr7(GRCh37):g.83796493_83796262 that is inserted at position Chr7(GRCh37):g.83796375. It is a deep intronic duplication located at NM_006080.2:c.112+27,298_c.112+27,529 in the cDNA.

It is inappropriate to list Laura, even with a footnote, as having the variant. We simply don't know. The variant was found in Kathleen, Caleb and Patrick. Caleb did not have an ECG.

Neither Kathleen or Patrick has electrocardiographic features of Brugada syndrome (see Prof Skinner's report). The evidence for this variant is limited but favours this being a benign variant.

PRKAG2 **(document paragraph 8.6.5 and following)**

The identified variant is a duplication of the segment Chr7(GRCh37):g.151325759_151325929 that is inserted at position Chr7(GRCh37):g.151325806. It is a deep intronic duplication involving the segment of at NM_016203.3:c.754+3,226_c.754+3,396.

Variants in this gene are associated with a highly penetrant, progressive cardiomyopathy which typically first presents in the teens or twenties, although onset may occur in the first decade of life and one patient has been reported with earliest symptoms aged two years (PMID:11748095). A pathogenic variant in this gene that had led to progression of disease sufficient to cause arrhythmia would be associated with very abnormal cardiac pathology. This was not identified at post mortem examination of any of the children's hearts. Kathleen Folbigg has had echocardiograms which showed no evidence of cardiomyopathy, and electrocardiograms which did not show any of the features associated with variants in *PRKAG2*.

This variant is thus definitively unrelated to the deaths of any of the children.

SCN1A **(variant provided only in the spreadsheet of structural variants)**

The identified variant is a deletion of the segment Chr2(GRCh37):g.166905667_166905743. It is within intron 6 (NM_01165963.1:c.965-208_c.965-284) and partially overlies the common Chr2(GRCh37):g.166905635_166905709del varaiant that is present in heterozygous form in 30% of non-Finnish Europeans and in homozygous form in 9.92%.

This variant is likely benign.

CALM2 **variant (document paragraph 8.8 and following)**

Note that we have reviewed this variant in detail in our report. Commentary is provided here (and in relation to the variant in *MYH6*) in order to note errors in the report by Professors Vinuesa and Cook which have led to misclassification.

As discussed in our report, variants in *CALM2* are associated with a severe, early-onset form of long QT syndrome. The fact that Kathleen Folbigg has reached her present age without signs of long QT syndrome represents strong evidence against this being a pathogenic variant. In relation to the specific criteria applied by Professors Vinuesa and Cook:

PM1: this criterion is incorrectly applied. The amino acid residue affected by the variant is not located within the calcium binding domains where the known pathogenic variants cluster. This point is explained (with a figure which demonstrates the point) in our report. It is also clearly shown in a figure in a paper referred to by Professors Vinuesa and Cook (Kotta et al, 2018 PMID: 30574507).

PM2: correct

PP1: this criterion is incorrectly applied. The criterion is "Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease". In order to apply this criterion there would need to be multiple members of the family clearly affected by severe long QT syndrome. There are no family members clearly affected by long QT syndrome. The available evidence is that Kathleen Folbigg does not have long QT syndrome (note Prof Skinner's report).

PP2: This is debatable. While the information used by Professors Vinuesa and Cook to apply this criterion is largely incorrect, a reasonable laboratory could apply this criterion using accurate information. Specifically, they state, incorrectly, that there are no missense benign variants described. In fact there are numerous known benign missense variants (see figure in our report). The measure pLI is irrelevant; this is a measure which refers to tolerance of a gene to loss of function variants, but the *CALM2* variant is a missense variant, not loss of function. However, gnomAD, the database which provides the pLI statistic, also provides data regarding the tolerance of a gene to missense variants (such as the variant identified here). The relevant score here is a z score, which in this case is 2.79. This z score ranges from -5 to 5; our laboratory's threshold for this score is >3, i.e. if a gene has a score lower than this we do not consider it as meeting this criterion. However, a reasonable laboratory could possibly use a less stringent criterion and apply PP2 for this gene.

PP3: correct

PP4: this criterion is incorrectly applied. The criterion is " Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology". SIDS (listed as the phenotype by Professors Vinuesa and Cook) is not a condition with a single genetic aetiology. Long QT syndrome is also not a condition with a single genetic aetiology, and in any case there is no evidence that any family member was affected by Long QT syndrome.

In their report, Professors Vinuesa and Cook conclude that there are "1(2) moderate and 4 supporting [pathogenic criteria]". In fact, at most there is one moderate (PM2) and two supporting criteria (PP2 if accepted, and PP3). They classify the variant as "VUS or Likely Pathogenic". VUS is the correct classification, even without applying the Benign criterion BS2 (observed in a healthy adult individual for a disorder with full penetrance expected at an early age). As discussed, it is appropriate to apply BS2.

In summary, there are multiple errors in the classification process for this variant which have led to undue weight being placed on the likelihood that it is pathogenic.

MYH6 **variant (document paragraph 8.9 and following)**

As discussed in our report, there is Limited evidence (by the Clingen criteria) linking variants in *MYH6* to a relevant phenotype. It is thus inappropriate to apply the ACMG criteria to a variant in this gene in relation to early onset cardiac arrhythmias, as Professors Vinuesa and Cook have done. However, even if there were strong evidence linking this gene to a relevant condition, there are errors in the application of pathogenicity criteria. Specifically:

PM1: The criterion is "Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation". At 8.9.3 there is discussion of the location of the variant near a known functional domain, but the critical point that this needs to be "without benign variation" is not considered. In fact, there is considerable benign variation in the vicinity of this variant, both affecting surrounding amino acids and Pro82 itself. The

latter include 4 alleles of Pro82Ser (the variant found in this family) as well as 28 alleles of Pro82Leu, a different change affecting the same amino acid. The nearest reported pathogenic variant (in relation to a different cardiac condition) is at some distance from Pro82. Thus, this criterion cannot be applied.

PM2: This is marginal. For such a rare condition, 4 alleles in gnomAD is more than would be expected, but it is still conceivable that this many alleles could be observed, and for this still to be a pathogenic variant. A reasonable laboratory could perhaps apply this criterion.

PP1: This criterion is incorrectly applied. See above for a discussion of the correct application of this criterion. There is no evidence of any cardiac phenotype which has been associated with *MYH6* variants in any family member. There is no basis for asserting that there is co-segregation of the variant with phenotype.

PP3: correct

PP4: This criterion is incorrectly applied. See above for a discussion of this point, which applies to the *MYH6* variant just as it does to the *CALM2* variant.

In summary, there are multiple errors in the classification process for this variant. Even if there were sufficient evidence to link this gene to a relevant phenotype, at most PM2 and PP3 could be applied. In their report, Professors Vinuesa and Cook interpret the variant as "Likely Pathogenic or VUS". There is no basis on which this variant could be interpreted as Likely Pathogenic.

***IDS* variant p.Pro47Ala (document paragraph 9.3 and following)**

We have very strong evidence that Patrick did not have Hunter syndrome, based on testing performed at the National Referral Laboratory in Adelaide (also known as the Dept of Chemical Pathology, Adelaide Children's Hospital at the time) (report dated 25/10/1990). These tests included a very sensitive semiquantitative measure of glycosaminoglycans, and qualitative assessment of mucopolysaccharides by high resolution electrophoresis. As the report states, both are normal and this essentially excludes the diagnosis of Hunter syndrome. Enzymology is not required in a patient with these results. In addition, multiple forensic pathologists have reviewed histopathology of relevant tissues from Patrick's post mortem examination and have not found evidence of abnormal storage.

We also know that even if Patrick had had Hunter syndrome, it would have been irrelevant to his cause of death, because the causes of death in Hunter syndrome relate to the consequences of advanced disease which develops over time. Such advanced disease is associated with ample clinical evidence that the child has a mucopolysaccharidosis. Seizures (discussed at 9.3.5) are feature of advanced cerebral disease of a type not present in Patrick.

Considering application of the the criteria in the report:

PM2: correct

PM5: correct

PP1: As discussed above, application of this criterion is incorrect. The variant was present in Patrick but he did not have Hunter syndrome, and there is no other person in the family with Hunter syndrome in whom the variant could co-segregate with phenotype.

PP2: Incorrect. Again, the pLI statistic has been incorrectly applied to this variant, which is a missense variant – not loss of function. *IDS* is actually tolerant to missense variation (Z score 1.58).

PP3: correct, although the reference in the comments to 'paper indicating severe misfolding' is irrelevant to this criterion.

PP4: incorrect. Again, the criterion is "patient's phenotype or family history is highly specific for a disease with a single genetic aetiology". Patrick had no features of Hunter syndrome, and had strong biochemical evidence against this diagnosis.

In summary, multiple errors in application of criteria as well as a failure to consider phenotypic and laboratory data have led to misclassification of the variant. This is formally a VUS, although given the strong evidence against pathogenicity it would be reasonable to classify the variant as Likely Benign.

***NLRP1* variant p.Gly881Arg (document paragraph 9.7.1 and following)**

NLRP1 is associated with Autoinflammation with arthritis and dyskeratosis. This rare disorder has clinicall manifestations of extensive regions of the skin covered with scaly, hyperkeratotic, purple-brown skin macules, polyarticular arthritis (involving wrists, knees, and ankles), immunological impairment with opportunistic infections and growth rretardation. There is no clinical evidence that Patrick had this disorder.

The reference to death in mice 'lacking' this gene seems to refer to homozygous null mice; this is a heterozygous VUS. Cardiac conditions in humans have not been described in association with variants in this gene.

***PTPN13* variant p.Gln716His (document paragraph 9.7.2 and following)**

There is no evidence linking variants in this gene to human Mendelian disease.

The mouse phenotype of increased resistance to infectious disease does not appear relevant.

Table at 8.10 and 11

Most of these variants are self-evidently irrelevant – heterozygous variants where the gene is associated with a recessive condition (i.e. carrier at most), condition irrelevant etc.

The variants in *MYPN* and *CHRNB2* could not be identified with the data provided and clarification has been requested.

We did not find any of these likely to be pathogenic.