**TITLE**

**Cardiomyopathies in 100,000 Genomes Project: interval evaluation improves diagnostic yield and informs strategies for ongoing gene discovery**

We thank the editor for their comments and corrections

There are hyperlinks in your manuscript text. We require these to be added to the reference list and cited accordingly throughout the text

We have changed these as requested and added them to the reference list.

We further require a statement describing whether the research conformed to the principles of the Helsinki Declaration.

We have added this to the ethics declaration.

Please ensure that tables s10 and s11 do not contain indirect identifiers that together may identify an individual (3 or more indirect identifiers) for which a consent to publish statement would be needed.

Thank you for raising this.

We have reviewed indirect identifiers, and can confirm that the data presented are not identifiable.

As a precaution, we have removed information on which parent a variant has been inherited from.

We note that all the tables have been exported through the Genomics England Airlock system and have been reviewed by a member of the Genomics England team prior to export. In addition the manuscript has been sent to Genomics England for review prior to submission.

Reviewer #1: The authors have mostly answered my concerns, however, Table S11 should include the cDNA and p. nomenclature for the variants (as Table S10 already has).

Many thanks to the reviewer for bringing this to our attention, we have now added the c. and p. variant nomenclature to Table S11.

Reviewer #2: The authors have mostly satisfactorily reviewed the article according to the recommendations from the reviewers. The addition of data for unsolved paediatric cases greatly improved the paper. However, to further improve readability, it would be important for the authors to be more clear on what the new table 3 exactly represents. Why are non cardiomyopathy genes includes? (e.g. LDLR , KCNQ1). In the same line, some genes listed in figure 6 are not related to the mentioned phenotype (PKP2 or TTN for HCM for example). Finally table 4 is divided into several pages, which made it very challenging to review.

Many thanks to the reviewer for their helpful comments. We have reworked the presentation to address these comments.

Table 3 presents genes that are not on the current UK paediatric or syndromic cardiomyopathy panel (R135 CM panel), but in which variants were considered to be diagnostic for patients with cardiomyopathy in 100KGP. There are 22 such genes. Table 3 distinguishes which of the 22 genes have an associated disease where cardiomyopathy is a recognised feature (dark purple) that could reasonably be included on a syndromic panel and those genes where cardiomyopathy is not known to be associated (light purple). For these latter genes it is not always possible from the information stored in GEL to work out why the lab concluded the case was solved.

The same 22 genes are highlighted in Figure 6 (bold dark and light purple). We have also highlighted (in grey) those genes that are associated with cardiomyopathy, but not robustly associated with the type of cardiomyopathy reported e.g. PKP2 and HCM. TTN was incorrectly presented as reported in an adult with HCM on Figure 6 which we have now amended.

The wording has been updated as follows:
*“Twenty-two CM cases where the recruiting GMC concluded the case to be solved involve genes not on the current R135 CM panel ‘green’ list (see Figure 6 genes in bold). Most of these genes (12/22) are associated with syndromic conditions where CM can be a feature but is unlikely to be found in isolation e.g. NEB related nemaline myopathy. In keeping with this it is mostly those with complex CM i.e. those who were recruited under a different disease category who have findings in these genes. Variants in these genes would have been tiered by GEL for analysis because the patient’s additional phenotype terms triggered other disease panels to be applied (see dark purple genes Table 3). Two of these twelve genes, NDUFA4 and FKRP, are already on the R135 CM panel ‘amber’ list. However, going forward it would be reasonable to include the other 10 genes on a syndromic paediatric CM panel and they may already be on more comprehensive gene lists used by laboratories outside of the UK.*

*In contrast, for 10/22 genes responsible for solved cases and not on the R135 CM panel, CM is only very rarely, or not known to be associated e.g. ANKRD11 related KBG syndrome or LDLR related familial hypercholesterolaemia, see Table 3. It is not possible from the information available in 100KGP to be sure why the recruiting centres concluded these CM cases were solved. These could be partial diagnoses where the CM phenotype remains unexplained, or the CM could be a secondary finding. In some instances, the reported CM could be an expansion of the known phenotype. Further input from the recruiting clinical centre will be needed to resolve these cases.*

*There are also cases documented as solved where the gene is on the CM panel, but not robustly associated with the type of CM reported e.g. PKP2 seen in HCM (see grey genes on Figure 6). Again, further input regarding the phenotype of the patient is needed to unravel these cases.”*

Finally, we will request that Table 4 be presented in landscape when it is published to make it easier to read.

Reviewer #3: The authors should be commended by the changes made in the analysis and the writing of the manuscript. The essence and novelty of their findings have become much more prominent.
I have some minor comments left for the authors:
1. I do not completely agree with the response to my first comment. I don't see how a P/LP variant in a novel gene has clinical implications when there is not information about prognosis or genotype-phenotype associations. There is a distinct difference between the classification of a variant as P/LP and the gene-disease validity. This might be better nuanced in the manuscript.

We thank the reviewer for taking the time to review the article again and for their comments.

We agree that there is a distinct difference between the classification of a variant as P/LP and the gene disease validity. As an example we note that GEL reported a patient with cardiomyopathy as ‘case solved’ with an *LDLR* pathogenic variant. While this variant explains the patient’s familial hypercholesterolaemia, it probably does not explain their cardiomyopathy and more likely represents a partial diagnosis. We wanted to highlight instances like this in the CM cohort. We are therefore not suggesting that the genes highlighted in Figure 6 are all potential novel CM genes but rather we wanted to explore why variants in these genes were considered diagnostic in patients with reported cardiomyopathy. We have now amended the wording describing Figure 6 and Table 3 to be clearer about what the highlighted genes represent. Please see updated wording above. Similarly in our re-analysis we have been cautious about reporting any of our findings as ‘definite’ diagnoses as we recognise that even a previously reported pathogenic variant will only be diagnostic in the context of the correct phenotype and this will need to be checked with the recruiting clinical centres.

With regard to the clinical implications of a P/LP variant in a novel gene, this may or may not have useful prognostic implications depending on the homogeneity of the phenotype reported in other cases. Furthermore, a molecular diagnosis would enable potential participation in clinical trials, cascade genetic testing for relatives, and reproductive options for both patient and relatives.

2. Would invasive prenatal testing (or PGT) be allowed for a gene without robust gene-disease validity?

No, in the UK prenatal testing (or PGT) would not be considered for a gene without robust gene-disease validity.

3. I'm still missing any recommendations for the clinical practice: should we broaden or gene panels? Only for specific patients? Should we re-evaluate the genetic testing for every patient with CM of 5 years ago? Should WGS be the standard now?

Thank you for raising this point. We have now included more details of our recommendations in the discussion and conclusion.

*‘In this study we identified several genes where CM is a known feature of the associated disease but these genes are not currently included on the R135 CM panel. It would be reasonable to add these genes to a syndromic CM panel. Specifically, for MAP3K7, CM can be a principle, if not the presenting, feature of the disease so this gene should be routinely evaluated. Overall, more work is needed to curate a comprehensive list of genes strongly associated with early onset cardiomyopathy…’*

*‘…We demonstrate the benefit of re-analysing their genomic data achieving a potential diagnostic uplift of 18%. The value of iterative re-analysis has been shown in other disease areas as well. However we recognise that incorporating this in routine clinical practice presents considerable practical challenges, and at present, it may largely be through research efforts that patients have their data re-evaluated…’*

*‘…Overall our findings support the use of genome sequencing (GS) over targeted panels and exome sequencing for paediatric CM testing where it is possible. In the NHS, GS is already standard practice for paediatric and syndromic CM. Importantly however, the scope of analyses varies considerably between laboratories, and is often restricted to a virtual panel.* *Better curation of gene disease relationships for paediatric and syndromic cardiomyopathy and ongoing sharing of clinical and research findings will help to standardize clinical panels and improve future variant identification.’*