Correspondence on "ACMG STATEMENT: ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG)" by Miller et al.

We were interested to read the recent update on recommendations for reporting of secondary findings in clinical sequencing[1], and the accompanying updated list of genes in which secondary findings should be sought (ACMG SF v3.0)[2]. Though the authors discuss challenges around incomplete penetrance in considerable detail, we are concerned that the recommendations do not fully convey the degree of uncertainty regarding the penetrance of variants in genes associated with inherited cardiomyopathies, which make up almost a quarter of the list. Since penetrance is incomplete and age-related, individuals found to carry variants will often require surveillance, rather than a one-off definitive diagnostic assessment. There is a lack of evidence regarding benefits, harms, and healthcare costs associated with opportunistic screening.

Here, we review the data from the studies cited to support the inclusion of two new dilated cardiomyopathy (DCM)-associated genes, *FLNC* and *TTN*, alongside other published data, and provide new analyses of publicly available data. Many of our conclusions may also be applicable to genes included in the previous ACMG SF v2.0. Of note, the ACMG/AMP standards have been calibrated for variants found in people with confirmed disease: we do not discuss here the further challenges in identifying which variants have disease-causing potential outside this context.

There are many challenges in assessing and reporting penetrance. Many penetrance estimates come from studies in families of affected individuals, where penetrance may be higher than in the wider population. Individuals found to carry (likely) pathogenic variants (P/LP) in genotype-first analyses can be considered in four groups: (a) known affected, (b) undiagnosed affected, (c) unaffected but will develop disease, (d) will never develop disease. Group (a) are, by definition, outside the scope of these

recommendations but they are often included in studies estimating penetrance, which is appropriate for some questions, but may over-estimate the benefits of opportunistic screening. Cohorts used in genotype-first analyses may also be enriched or depleted for this group according to ascertainment approach. One-off assessment will detect (b) but not (c), for whom burdensome and costly longitudinal surveillance may be required.

FLNC

"*The SFWG voted to include this gene based on its high penetrance, severity of the phenotype if untreated, and the strong potential benefit of intervention based on returning P/LP variants in this gene as a SF*"[2]. While we agree with the comments on apparent phenotypic severity in *FLNC*-related DCM, we are not aware of any data from population studies to justify an assertion of high penetrance outside of families with known disease. The recommendations cite a family-based analysis[3], a cardiomyopathy case series[4], and a review article[5]. The review authors note that *"the finding of a truncating FLNC variant in otherwise healthy subjects outside of a familial context is much less clear at the moment, as there is not enough knowledge regarding penetrance, expression, and clinical correlation*"[5].

We therefore performed an analysis in 200,581 UK Biobank (UKBB) participants with exome sequencing data available (median age 58 at recruitment). We identified 50 individuals heterozygous for 38 rare truncating variants in *FLNC* (FLNCtv*;* prevalence 0.025%) that would be considered P/LP in an individual with DCM. The prevalence is comparable to gnomAD (47 heterozygous individuals in 125,408 = 0.037%). Among these 50 participants, there were no cases of DCM, or other inherited cardiomyopathy, and DCM was not identified in the five individuals with cardiac MRI, which we have previously found to have higher sensitivity than ICD codes alone[6]. Lifetime risk of major adverse cardiovascular events (MACE, see Supplementary Material) was higher in *FLNC* variant heterozygotes (HR=1.9, P=0.04), driven by increased risk of atrial fibrillation and arrhythmia (HR=2.4,

P=0.0096), but with modest absolute increase. There were five deaths, three heart failure events, and no cardiac arrests, in 569 person-years of follow-up, which was not significantly different from the rest of the population (Table S2).

TTN

The authors of the recommendations found that "*new evidence indicated significant risk for cardiomyopathy among those with TTN truncating variants (*TTNtv*)*"[2], citing a study of two cohorts drawn from health systems[7]. The prevalence of DCM in the cohorts was higher than population estimates, consistent with ascertainment on the basis of disease, as might be expected in a health system, and as reported by the study authors[7]. The proportion of TTNtv+ individuals who manifested DCM in these cohorts was 30% and 7.5%, which is likely an over-estimate of penetrance in an unselected population. Incident cases were not reported.

In UKBB we identified 877 participants (0.44%) carrying one or more of 487 rare TTNtv that would be reportable, similar to previous estimates[8]. We estimated the prevalence of known cardiomyopathy in TTNtv heterozygotes as 1.4% at enrolment (excluding coronary disease and HCM; Table S2). These participants with known disease may benefit from a molecular diagnosis reported as a secondary finding if not already tested.

Amongst those TTNtv heterozygotes not coded with cardiomyopathy who underwent cardiac MRI, 2.4% met criteria for DCM. This estimates the yield of a one-off cardiac assessment following reported secondary findings.

A further 3.4% TTNtv heterozygotes developed cardiomyopathy subsequently to the 1.4% at enrolment (Table S2), yielding ~3 incident cases per 1,000 person-years of surveillance (Figure S1), consistent with previous reports[8]. This estimates the yield of ongoing surveillance in those not diagnosed at first assessment, the costs and harms of which have not been well characterised to our knowledge. We

observe an increased lifetime risk of MACE in TTNtv heterozygotes (HR=2.6, P=<0.001), driven by increased risk of atrial fibrillation and arrhythmia (HR=2.7, P=<0.001), HF (HR=4.4, P=<0.001), and CM (HR=15.0, P=<0.001), albeit with a small absolute increase and no significant difference in death, cardiac arrest, or stroke, with a total of 141 MACE during 10,132 person-years follow-up.

Estimating mortality in TTNtv-associated DCM as ~4% over 4 years[9,10], and modelling this as entirely preventable with diagnosis and treatment, we could estimate ~8,000 person-years of surveillance (1,600 CMR scans if 5-yearly imaging) would yield 25 new diagnoses of DCM, with an opportunity to prevent 1 death over the subsequent 4 years (Figure S2).

Alternatively, if we estimate the total excess mortality in TTNtv heterozygotes as 1% (over 10 years) and assume this would be fully preventable by return of secondary findings followed by long-term surveillance, then we would need to enrol 100 people into long-term surveillance to prevent one death (Supplemental Methods), even in this over-optimistic scenario.

We acknowledge the likelihood of survivorship bias in the UKBB that may skew lifelong penetrance estimates. However, the prevalence of DCM is close to population estimates (Table S1), which speaks against a substantial depletion of cases. UKBB is likely to provide reasonable estimates for opportunistic screening carried out in adults – e.g., in those undergoing sequencing for adult-onset breast or other cancers – since opportunistic screening is performed in those who did not manifest the screened-for disease earlier in life. Furthermore, an important proportion of individuals undergoing clinical sequencing will be healthy adult parents of children with rare diseases, sequenced for trio analyses.

The primary diagnoses in those undergoing clinical sequencing may also carry an adverse prognosis with competing risks that further reduce the benefits of cardiac screening and surveillance.

In summary, there is much uncertainty regarding the penetrance of variants in *FLNC* and *TTN* that can cause DCM. We do not believe that an assertion of high penetrance is justified for *FLNC*. The authors of

the new recommendations acknowledge that TTNtv have low penetrance, but we provide further data to illustrate the yield of surveillance in individuals not known to have disease at first assessment. We think it is premature to recommend *TTN* screening, and thereby make this the standard-of-care, given that the costs, harms, and benefits are not yet well characterised.

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COMPETING INTERESTS

J.S.W. has consulted for MyoKardia, Inc. and Foresite Labs. D.P.O. has consulted for Bayer.

ADDITIONAL INFORMATION

Supplementary information online

Correspondence and requests for materials should be addressed to JSW.

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Data availability

The UK Biobank biomedical database can be accessed globally for public health research. The data supporting the findings of this Correspondence are presented in the Supplementary information.

CRediT statement

Conceptualization: J.S.W; Data curation: K.A.M., S.L.Z., A.H., K.J., M.E., A.D.; Formal Analysis: K.A.M., S.L.Z.; Supervision: J.S.W., D.P.O., T.R.L., A.R.; Writing – original draft: K.A.M., J.S.W.; Writing – review & editing: S.L.Z., A.H., K.J., M.E., A.D., N.W., A.R., T.R.L., D.P.O.

Ethics declaration

The UK Biobank study (https://www.ukbiobank.ac.uk; PMID 25826379) was reviewed by the National Research Ethics Service (11/NW/0382, 21/NW/0157). Written informed consent was required and the

study adheres to the principles set out in the Declaration of Helsinki. The data was de-identified. This study was conducted under terms of access approval number 47602.