**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.**

**Supplementary Information**

## Methods

*The UK Biobank cohort*

The UK Biobank (UKBB) recruited 500,000 participants aged 40–69 years across the UK between 2006 and 2010 (National Research Ethics Service, 11/NW/0382, 21/NW/0157). Written informed consent was provided. This study was conducted under terms of access approval number 47602. 200,581 UKB participants underwent exome sequencing[1] and 21,129 of those had cardiac MRI imaging available for analyses.

*Exome sequencing data analysis and variant curation*

Variants within 100 bp of coding regions of the *TTN* and *FLNC* genes were extracted from the exome sequencing data. We identified truncating variants (tv) predicted to introduce a premature termination codon (often described as loss-of-function (LoF), i.e., frameshift, essential splice, and stop gained variants) that had a MAF of <0.1% in gnomAD and UKBB and a max FAF <0.0084%[2] in gnomAD. TTNtv were annotated with the cardiac expression of the exon impacted and variants affecting exons constitutively expressed in the heart (PSI>90%[3]) were retained. The exome sequencing data was annotated using Ensembl Variant Effect Predictor (VEP; version 104) with a plugin for gnomAD (version r2.1) and LOFTEE, and the data was organised using PLINK (version 1.90p 64-bit). The VEP output was analysed using R (version 3.6.0) and Rstudio (version 1.3.1073). Variants that were predicted by LOFTEE to be low confidence LoF variants were excluded from analyses.

*FLNC variants and DCM*

*FLNC* variants have been associated with both HCM and DCM, alongside other disorders. FLNCtv have been associated with DCM. While some missense variants are associated with HCM (especially in the ROD2 domain), there is only one missense variant observed in the UKBB that has been previously classified as P/LP for DCM, carried by eight UKBB participants (c.318C>G)[4]. This variant was originally reported in a compound heterozygous state[5] and there is not compelling evidence that this variant in the heterozygous state would be P/LP for DCM (ClinVar ID 267289). Furthermore, no other protein altering variants have evidence for DCM causation in ClinVar. Our analyses therefore focused on FLNCtv.

While FLNCtv are associated with DCM, with data suggesting an adverse clinical course, and FLNCtv appear penetrant when ascertained in families with DCM, penetrance has not been characterised in individuals not selected for DCM or a family history of disease.

*UKBB codes and datasets for phenotype analysis*

First occurrence data:

The UKBB provided first occurrence of health outcomes defined by 3-character ICD10 code using Hospital in-patient records, Death records, and Primary care records. The 'first occurrence' fields define each health outcome by the 3-character codes within ICD10's diagnostic chapters and provides the earliest date that the 3-character ICD10 (or mapped codes) was recorded through either self-report at any assessment centre, inpatient hospital data, primary care or death record data. Hospital inpatient data does not record the diagnosis date directly, but rather information about the dates the hospital episode started and ended and the dates the hospital admission started and ended. The episode start date has been used as the best proxy for the diagnosis date. If this was missing, the admission date, episode end date or discharge date were used instead. Date of the event was recorded in primary care

data. Self-report gives the interpolated year when non-cancer illness first diagnosed, obtained during verbal interview at the UKBB assessment centre.
The fields analysed were as follows:

- Any cardiomyopathy (CM): I42* excluding reported hypertrophic CM (HCM) or coronary artery disease (CAD) at 2021 data release (below) - an inclusive group of non-ischaemic & non-hypertrophic cardiomyopathic phenotypes, intended to maximise sensitivity to detect individuals with phenotypes potentially attributable to variants in *FLNC* or *TTN*
- Cardiac arrest: I46*.
- Atrial fibrillation and flutter/arrhythmia: I48* and I49*.
- Stroke: I64*.
- Heart Failure: I50*.

Date of death:
The UK Biobank receives death notifications on a regular basis through linkage to national death registries and provides the date of death.

2021 data release of refreshed Hospital Episode Statistics (HES) plus Self-Reported data:
The following codes were used to identify patients of the following conditions, with broad conditions matching the first reported data above:

- Dilated cardiomyopathy (DCM): ICD10 I420, I426, I427, O903; excluding HCM and coronary disease.
- Hypertrophic cardiomyopathy (HCM) [for exclusions]: Self-reported 1588; ICD10 I422, I421; ICD9 4251.
- Coronary disease [for exclusions]: Self-reported 1075, 1070, 1095, 1523; ICD10 I210, I211, I212, I213, I214, I219, I220, I221, I228, I229, I252; ICD9 410, 411, 412; Procedure K483, K491, K492, K493, K494, K498, K499, K751, K752, K753, K754, K758, K759, K401, K402, K403, K404, K408, K409, K411, K412, K413, K414, K418, K419, K421, K422, K423, K424, K428, K429, K431, K432, K433, K434, K438, K439, K441, K442, K448, K449, K451, K452, K453, K454, K455, K456, K458, K459.
- Coronary artery disease (CAD) [for Non-ischaemic cardiomyopathy definition]: ICD10 I21*, I22*, I23*, I23*, I241, I252, I255, I256; Procedure K40*, K41*, K42*, K43*, K44*, K45*, K46*, K47*, K48*, K49*, K50*
- Structural heart disease (HD) [for Non-ischaemic cardiomyopathy definition]: ICD10 I05*, I06*, I07*, Q20*, Q21*, Q22*, Q23*, Q24*, Q25*, Q26*; Procedure K25*, K26*, K27*, K28*, K29*, K30*, K31*, K32*, K34*, K36*, K37*, K38*
- Left ventricular systolic dysfunction (LVSD) [for Non-ischaemic cardiomyopathy definition]: ICD10 I420, I426, I427, I255, I502; Procedure K617

Non-ischaemic cardiomyopathy:
To obtain an upper estimate of variant penetrance, we used an inclusive definition of non-ischaemic cardiomyopathy (including hypokinetic non-dilated CM) defined as HF with left ventricular systolic dysfunction (LVSD) and without CAD or structural heart disease (HD). Phenotyping was performed using a rule-based algorithm based on ICD-10 and procedure codes in UKBB linked HES data and left ventricular ejection fraction (LVEF) derived from Cardiac Magnetic Resonance imaging (CMR) was used in the calculation as binary classifier for LVSD[6]. Participants were identified as cases if they did not have CAD or structural HD and had LVSD. Participants were excluded from the analysis if they had CAD or structural HD, or if they had: 1) HF without history of LVSD or 2) CAD or structural HD. LVSD was defined as LVEF <50 or the presence of LVSD codes.

Analysis of cardiac MRI data:
Summary CMR data was analysed as previously described[6]. Participants were flagged for DCM if they had increased LV end-diastolic volume (LVEDV) (women >175mL and men >232mL[7] with LVEF <50% and no reported HCM or coronary disease.

Disease definitions:
We used inclusive definitions of cardiomyopathy using the data described in this section, which will tend to provide upper estimates of the utility of opportunistic screening and surveillance. We use four approaches to identify CM:

1) Primary analyses use the "first occurrence" data

The first occurrence data provided the earliest date that an event or diagnosis of interest was reported for the UKBB participants. This data allowed for a time-specific assessment of the incidence of diagnoses; e.g. presence of a diagnosis after the date of recruitment up to the date of imaging.

2) Cardiac MRI imaging data

The diagnosis of participants with DCM via MRI imaging that do not have a previous diagnosis of DCM, allows for the identification of individuals that would be detected in a one-off assessment.

3) Hospital episode statistic data

The HES data is similar to 1) but it is not time-specific and allows for a DCM coded-specific analysis.

4) Non-ischaemic cardiomyopathy data

This inclusive definition of non-ischaemic cardiomyopathy allows for the identification of the maximum UKB participants that may present with non-ischaemic cardiomyopathy.

*Statistical analysis*
The association between variant carrier status and diagnoses were tested using Chi-squared or Fisher's exact tests. Lifetime survival analysis was performed with major adverse cardiovascular events (MACE) and death as the primary outcome and hazards ratios estimated and graphically presented as cumulative hazards curves using the *survival* and *survminer* packages in R. MACE consisted of heart failure (including cardiomyopathy that excluded HCM and coronary disease), stroke, atrial fibrillation or arrhythmia, and cardiac arrest.

*Calculation of the Number Needed to Treat (NNT)*
Although there was no significant difference in death based on TTNtv carrier status (P>0.05), the NNT was calculated based on the inverse of the adverse risk reduction of all-cause mortality (Table S1). The ARR was 0.9%, resulting in an NNT of 111.

*Estimation of background mortality*
Background mortality (Figure S2) was estimated as the mean death rate in 2019 of registered deaths in 59–69-year-olds in the UK by the Office for National Statistics (Reference number 12663). Counts of the number of UK male and female death registrations were summed for each single year of age, as were the mid-year population estimates for each single year of age. The death rate in 2019 for each year of age was calculated by dividing the counts of death for a single year of age by the corresponding population size. Mean death rate of 0.093% for 59-69-year-olds was calculated from the average of the resulting death rate values.

## Results

*Variants identified*

The 38 variants in *FLNC* were predicted by VEP to cause the following consequences to the resulting protein: frameshift variant (n=14); splice acceptor variant (n=4); splice donor variant (n=4); stop gained (n=16).

The 487 variants in *TTN* were predicted by VEP to cause the following consequences to the resulting protein: frameshift variant (n=211); splice acceptor variant (n=15); splice donor variant (n=44); stop gained (n=209); stop gained, frameshift variant (n=8).

*Lifetime risk*

Lifetime risk of MACE was increased in *FLNC* heterozygotes (FLNCtv: n=10 (20%), no FLNCtv: n=21,802 (11%); HR=1.9, 95%CI=1.04-3.6, P=0.04), driven by increased risk of atrial fibrillation and arrhythmia (FLNCtv: n=9 (18%), no FLNCtv: n=16,294 (8%); HR=2.4, 95%CI=1.2-4.6, P=0.0096), with no significant difference in death or HF (P>0.05).

Lifetime risk of MACE was significantly increased in TTNtv heterozygotes (TTNtv: n=221 (25%), no TTNtv: n=21,591 (11%); HR=2.6, 95%CI=2.3-3.0, $P=2.29x10^{-46}$), driven by increased risk of atrial fibrillation and arrhythmia (TTNtv: n=171 (20%), no TTNtv: n=16,132 (8%); HR=2.7, 95%CI=2.3-3.1, $P=1.67x10^{-37}$), HF (TTNtv: n=99 (11%), no TTNtv: n=5,634 (3%); HR=4.4, 95%CI=3.6-5.3, $P=5.37x10^{-48}$), and CM (TTNtv: n=42 (5%), no TTNtv: n=663 (0.3%); HR=15.0, 95%CI=11.0-20.5, $P=7.27x10^{-65}$) with no significant difference in death, cardiac arrest, and stroke (P>0.05).

*Follow-up*

TTNtv heterozygotes (n=877) were followed up for 10,132 person-years, with a mean of 11.55 years per person. *FLNC* heterozygotes (n=50) were followed up for 569 person-years, with a mean of 11.39 years per person.

**Tables**

**Table S1 a) Counts of CM and MACE phenotypes, and death, and estimates of CM prevalence, reported for the individuals of UKBB with exome sequencing data and a subset with imaging data available for analysis; b) Corresponding P-values of the burden analysis comparing heterozygotes to the rest of the population.**

See excel document.

Prevalence of disease was estimated using diagnostic codes at 3-time points; enrolment, date of imaging, and at the most recent assessment. This was completed to mimic the participants that would be identified by secondary findings; known affecteds at recruitment, unrecognised affecteds identified at imaging, and heterozygotes that developed disease during follow up. At the time of imaging, we assessed prevalence using diagnostic codes & imaging definition. Incident cases were identified between these time points. The association between variant carrier status and diagnoses were tested using Chi-squared (normal coloured cell) or Fisher's exact (pink cell) tests. Non-significant associations were highlighted in red font. *, for each trait the total needs subtraction of participants identified at previous incidence(s).

**Table S2 Prevalence of DCM and TTNtv in Published Population Cohorts**

The proportion of each cohort with TTNtv were between 0.4%-1.4% across three population cohorts. The prevalence of DCM in each cohort was in the range 0.05%-6%, consensus estimates are 0.4% in literature[8]. The proportion of the cohorts with DCM and TTNtv was 1.5%-30.3%. *cohort number differs in article text; presented is number from Table 1[9].

| Reference | Cohort | Participants (n) | TTNtv (PSI>90) in cohort (n) | DCM in cohort (n) | Proportion of TTNtv heterozygotes with DCM (n) |
|---|---|---|---|---|---|
| Haggerty et al. 2019[9] | PennMedicine BioBank | 10,289* | 142 (1.38%) | 613 (5.96%) | 43 (30.28%) |
| Haggerty et al. 2019[9] | Geisinger MyCode Community Health Initiative | 61,040 | 359 (0.59%) | 622 (1.02%) | 27 (7.52%) |
| Pirruccello et al. 2020[10] | UK Biobank baseline DCM | 49,944 | 227 (0.45%) | 26 (0.05%) | 4 (1.76%) |
| Pirruccello et al. 2020[10] | UK Biobank new DCM | 45,747 | 196 (0.43%) | 26 (0.06%) | 3 (1.53%) |

## Figures

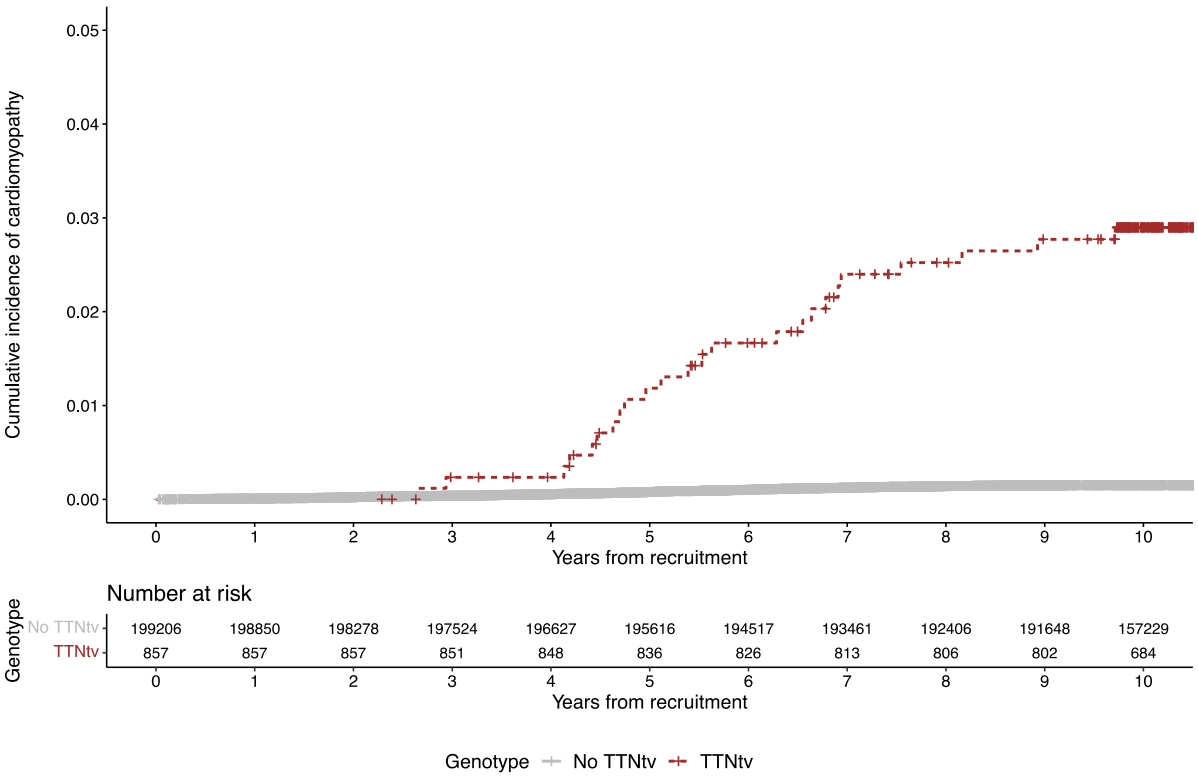


**Figure S1 Cumulative incidence curve of cardiomyopathy over ten years post recruitment to the UKBB, stratified by TTNtv carrier status**.
There were 2.8 events per 1,000 person-years. Individuals with cardiomyopathy and a code of HCM or coronary artery disease at any time (n=334; n=8 in TTNtv group) and individuals with cardiomyopathy at baseline (n=184; n=12 in TTNtv group) were excluded from the analysis.

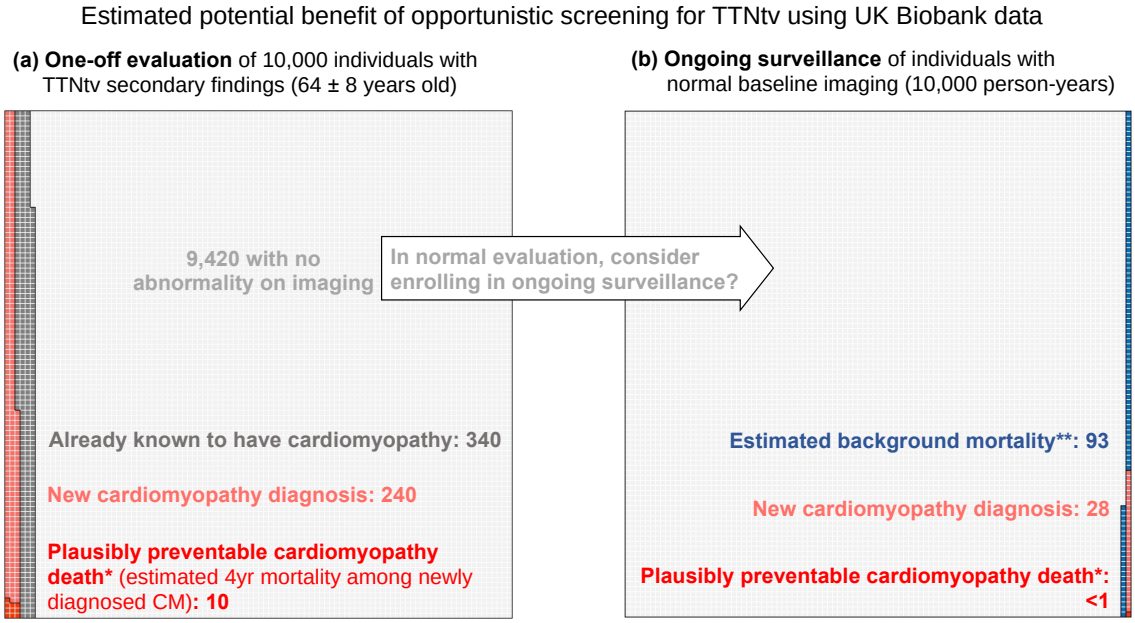


**Figure S2 Visual representation of the potential benefits of opportunistic screening for TTNtv estimated using UK Biobank data.**

A) Expected findings for a one-off cardiac imaging evaluation of 10,000 individuals with a TTNtv identified as a secondary finding. We estimate that 340 individuals would already be known to have cardiomyopathy, and 240 would be newly identified. CM-related mortality has been estimated as ~4%/4years[11,12], so we expect ~10 deaths in this time-frame amongst the newly identified cases, which might plausibly be preventable. This is one estimate of the benefit of opportunity screening, though it is not yet known to what extent early identification would prevent these, particularly given that this population will have other competing risks. Since they are undergoing clinical sequencing for another indication, the morbidity and mortality of that condition would influence the overall value of screening for secondary findings.

B) For the 9,420 individuals with no abnormality on initial imaging, we need to consider the potential value of ongoing surveillance, e.g., with serial imaging. For each 10,000 person-years of surveillance we expect 28 new cardiomyopathy diagnoses, with <1 plausibly preventable cardiomyopathy-related death in the next four years (based on mortality rates described above). For comparison, the estimated background mortality in the UK is 93 in 10,000 person-years. The background mortality in a population undergoing clinical sequencing for another indication may be higher.

These estimates are based on the UK biobank population, a population of older adults around the typical age of presentation for dilated cardiomyopathy. The yields of one-off and serial evaluation might be expected to be lower in younger individuals.

The potential benefit of opportunistic screening for sudden cardiac arrest prevention can also be estimated by directly measuring the incidence of sudden cardiac arrest. Amongst TTNtv heterozygotes in the UK biobank (including individuals already known to have disease, as well as those newly recognised) the incidence is 5/10,132 person-years, i.e., 0.05%, compared with a background SCA incidence of 0.04% in the remainder of the cohort (P>0.05).

*Plausibly preventable cardiomyopathy mortality was estimated as 4%/4yr among newly diagnosed cardiomyopathy[11,12]. **mean death rate in 2019 of registered deaths in 59-69-year-olds in the UK by the Office for National Statistics. The data supporting this figure can be found in the supplementary methods, Table S1, and Figure S1. Of note, these estimates do not account for competing risks.

**References**


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