

ASSOCIATION STUDIES ARTICLE

# Genome-wide association study of cardiac troponin I in the general population

Marta R. Moksnes<sup>1,†,\*</sup>, Helge Røsjø<sup>2,3,†</sup>, Anne Richmond<sup>4,†</sup>, Magnus N. Lyngbakken<sup>3,5</sup>, Sarah E. Graham<sup>6</sup>, Ailin Falkmo Hansen<sup>1</sup>, Brooke N. Wolford<sup>7</sup>, Sarah A. Gagliano Taliun<sup>8,9,10,11</sup>, Jonathon LeFaive<sup>10,11</sup>, Humaira Rasheed<sup>1,12</sup>, Laurent F. Thomas<sup>1,13,14,15</sup>, Wei Zhou<sup>7,16,17,18</sup>, Nay Aung<sup>19,20,21</sup>, Ida Surakka<sup>6</sup>, Nicholas J. Douville<sup>22</sup>, Archie Campbell<sup>23</sup>, David J. Porteous<sup>23</sup>, Steffen E. Petersen<sup>19,20,21</sup>, Patricia B. Munroe<sup>19,20</sup>, Paul Welsh<sup>24</sup>, Naveed Sattar<sup>24</sup>, George Davey Smith<sup>12</sup>, Lars G. Fritsche<sup>10,11</sup>, Jonas B. Nielsen<sup>1,6,25,26</sup>, Bjørn Olav Åsvold<sup>1,27,28</sup>, Kristian Hveem<sup>1,27</sup>, Caroline Hayward<sup>4,‡</sup>, Cristen J. Willer<sup>1,6,7,29,‡</sup>, Ben M. Brumpton<sup>1,12,30,‡</sup> and Torbjørn Omland<sup>2,3,5,‡</sup>

<sup>1</sup>Department of Public Health and Nursing, K.G. Jebsen Center for Genetic Epidemiology, NTNU - Norwegian University of Science and Technology, 7491 Trondheim, Norway, <sup>2</sup>Division of Research and Innovation, Akershus University Hospital, 1478 Lørenskog, Norway, <sup>3</sup>Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, 0316 Oslo, Norway, <sup>4</sup>MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh EH4 2XU, UK, <sup>5</sup>Division of Medicine, Department of Cardiology, Akershus University Hospital, 1478 Lørenskog, Norway, <sup>6</sup>Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA, <sup>7</sup>Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI 48109, USA, <sup>8</sup>Faculty of Medicine, Université de Montréal, Montréal, QC H3T 1J4, Canada, <sup>9</sup>Montréal Heart Institute, Montréal, QC H1T 1C8, Canada, <sup>10</sup>Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI 48109, USA, <sup>11</sup>Center for Statistical Genetics, University of Michigan School of Public Health, Ann Arbor, MI 48109, USA, <sup>12</sup>MRC Integrative Epidemiology Unit, University of Bristol, Bristol BS8 2BN, UK, <sup>13</sup>Department of Clinical and Molecular Medicine, NTNU - Norwegian University of Science and Technology, 7491 Trondheim, Norway, <sup>14</sup>BioCore - Bioinformatics Core Facility, NTNU - Norwegian University of Science and Technology, 7491 Trondheim, Norway, <sup>15</sup>Clinic of Laboratory Medicine, St. Olavs Hospital, Trondheim University Hospital, 7491 Trondheim, Norway, <sup>16</sup>Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA 02114, USA, <sup>17</sup>Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA, <sup>18</sup>Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA, <sup>19</sup>William Harvey Research Institute, Queen Mary University of London, Charterhouse Square,

<sup>†</sup>The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors.

<sup>‡</sup>The authors wish it to be known that, in their opinion, the last four authors should be regarded as joint Last Authors.

Received: January 5, 2021. Revised: April 23, 2021. Accepted: April 27, 2021

© The Author(s) 2021. Published by Oxford University Press.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

London EC1M 6BQ, UK, <sup>20</sup>National Institute for Health Research Barts Cardiovascular Biomedical Research Centre, Queen Mary University of London, London E1 4NS, UK, <sup>21</sup>Barts Heart Centre, St. Bartholomew's Hospital, Barts Health NHS Trust, London EC1A 7BE, UK, <sup>22</sup>Department of Anesthesiology, University of Michigan, Ann Arbor, MI 48109, USA, <sup>23</sup>Medical Genetics Section, CGEM, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK, <sup>24</sup>Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow G12 8TA, UK, <sup>25</sup>Department of Epidemiology Research, Statens Serum Institute, 2300 Copenhagen, Denmark, <sup>26</sup>Department of Cardiology, Copenhagen University Hospital, 2100 Copenhagen, Denmark, <sup>27</sup>Department of Public Health and Nursing, HUNT Research Centre, NTNU - Norwegian University of Science and Technology, 7600 Levanger, Norway, <sup>28</sup>Department of Endocrinology, St. Olavs Hospital, Trondheim University Hospital, 7006 Trondheim, Norway, <sup>29</sup>Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109, USA and <sup>30</sup>Clinic of Thoracic and Occupational Medicine, St. Olavs Hospital, Trondheim University Hospital, 7006 Trondheim, Norway

\*To whom correspondence should be addressed at: Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Norway. Postbox 8905, MTF5, 7491 Trondheim, Norway. Tel: +47 97036766; Email: marta.r.moksnes@ntnu.no

## Abstract

Circulating cardiac troponin proteins are associated with structural heart disease and predict incident cardiovascular disease in the general population. However, the genetic contribution to cardiac troponin I (cTnI) concentrations and its causal effect on cardiovascular phenotypes are unclear. We combine data from two large population-based studies, the Trøndelag Health Study and the Generation Scotland Scottish Family Health Study, and perform a genome-wide association study of high-sensitivity cTnI concentrations with 48 115 individuals. We further use two-sample Mendelian randomization to investigate the causal effects of circulating cTnI on acute myocardial infarction (AMI) and heart failure (HF). We identified 12 genetic loci (8 novel) associated with cTnI concentrations. Associated protein-altering variants highlighted putative functional genes: *CAND2*, *HABP2*, *ANO5*, *APOH*, *FHOD3*, *TNFAIP2*, *KLKB1* and *LMAN1*. Phenome-wide association tests in 1688 phecodes and 83 continuous traits in UK Biobank showed associations between a genetic risk score for cTnI and cardiac arrhythmias, metabolic and anthropometric measures. Using two-sample Mendelian randomization, we confirmed the non-causal role of cTnI in AMI (5948 cases, 355 246 controls). We found indications for a causal role of cTnI in HF (47 309 cases and 930 014 controls), but this was not supported by secondary analyses using left ventricular mass as outcome (18 257 individuals). Our findings clarify the biology underlying the heritable contribution to circulating cTnI and support cTnI as a non-causal biomarker for AMI in the general population. Using genetically informed methods for causal inference helps inform the role and value of measuring cTnI in the general population.

## Introduction

Cardiac troponins I (cTnI) and T (cTnT) are structural proteins involved in cardiac muscle contraction. During acute myocardial ischemia, cardiac troponin proteins are released into the blood stream and are used to diagnose acute myocardial infarction (AMI) (1). In AMI, cardiac troponin release is a result of direct ischemic cardiomyocyte injury. In contrast, the pathobiology of cardiac troponin release outside of AMI is not known, although low-level cardiac troponin elevations are associated with future cardiovascular events in the general population, leading some clinicians to suggest a need for wider use of cardiac troponins in risk stratification in general (2). Hence, a better understanding of low-level cardiac troponin release is needed, and the study of large population-based cohorts with genetic data could bring new insights.

The genetic determinants of cardiac troponin in the general population have not been studied in detail, and the downstream biology, including possible causal effects on cardiovascular disease, is unclear. The cTnI and cTnT proteins are encoded by the *TNNI3* and *TNNT2* genes (3), respectively, and mutations in these genes are recognized as disease-causing mutations in patients with familial cardiomyopathies (4,5). Patients with cardiomyopathies demonstrate increased concentrations of

circulating cardiac troponins (6), and these conditions are strongly associated with heart failure (HF) development (7). We recently demonstrated a close association between cTnI and left ventricular hypertrophy (8), which represents an intermediate stage prior to symptomatic HF (9). Moreover, as experimental studies have found immune responses targeting cTnI to aggravate acute cardiac damage and to induce myocardial dysfunctions and HF (10,11), cTnI may have a direct or indirect causal effect on HF development in humans. Circulating cardiac troponin concentrations are also influenced by protein degradation and clearance (12). A previous genome-wide association study (GWAS) in the general population from the Generation Scotland Scottish Family Health Study (GS:SFHS) reported five genetic loci associated with cTnI concentrations and four other loci associated with cTnT concentrations (13), however without replication in an independent cohort. More knowledge about the genetic determinants of circulating cardiac troponins could reveal new pathobiology important for HF development. In contrast, as AMI type 1 is a result of an acute epicardial coronary artery occlusion and cTnI is only expressed in cardiac myocytes, no causal role for cTnI is expected for incident AMI. Due to limited knowledge of the genetic contribution to circulating cardiac troponins, no previous studies have used genetically

informed methods of causal inference, namely Mendelian randomization (MR), to assess the causal effect of cardiac troponin concentrations on AMI or HF (14).

High-sensitivity cTnI has been cross-sectionally and repeatedly measured in three surveys of the Trøndelag Health Study (15) (HUNT) from 1995 to 2019. Additionally, recent efforts to sequence 53831 diverse genomes in the NHLBI TOPMed Program (16) have enabled the imputation of more than 26 million variants in HUNT and GS:SFHS combined, including less common loss-of-function variants that have not been previously investigated (13).

In order to validate previous findings and discover novel genetic variants associated with circulating cTnI in the general population, we conducted a genome-wide meta-analysis of cTnI concentration in 48 115 individuals from HUNT and GS:SFHS. Additionally, we assessed changes in cTnI with time in 5178 HUNT participants with repeated measures. Finally, we performed MR to investigate the causal effects of circulating cTnI on AMI and HF.

## Results

### Discovery of genetic loci associated with cTnI

After a discovery genome-wide association meta-analysis of HUNT ( $n=29839$ ) and GS:SFHS ( $n=18276$ ), we identified 12 genetic loci associated ( $P\text{-value} < 5 \times 10^{-8}$ ) with cTnI concentrations (Table 1; Supplementary Material, Tables S1 and S2, Supplementary Material, Figs S1 and S2). All 12 loci were well imputed in both HUNT and GS:SFHS and had consistent directions and effect sizes across both studies. Eight of the 12 loci were previously unreported, while 4 were previously reported, but not yet replicated (13). Additionally, we discovered 11 novel, rare variants ( $MAF < 0.001$ ,  $P\text{-value} < 5 \times 10^{-8}$ ) that were only imputed in HUNT and could not therefore be replicated (Supplementary Material, Table S1). Excluding individuals with a known history of comorbidities did not materially change the results (Supplementary Material, Table S4). No genetic loci were genome-wide significant ( $P\text{-value} < 5 \times 10^{-8}$ ) for changes in cTnI concentrations with time; however, three loci were suggestive ( $P\text{-value} < 10^{-6}$ ) (Supplementary Material, Tables S3 and S5).

We found three protein-altering index variants in cTnI loci: rs7080536 ( $MAF=0.04$ , effect size =  $-0.10$ ,  $P\text{-value} = 1 \times 10^{-12}$ ) in *HABP2*, rs1801690 ( $MAF=0.06$ , effect size =  $0.10$ ,  $P\text{-value} = 1 \times 10^{-14}$ ) in *APOH* and rs7481951 ( $MAF=0.41$ , effect size =  $0.05$ ,  $P\text{-value} = 2 \times 10^{-19}$ ) in *ANO5*. Furthermore, we identified a rare protein-altering index variant (rs151313792,  $MAF=0.004$ , effect size =  $0.26$ ,  $P\text{-value} = 1 \times 10^{-8}$ ) in *FHOD3*. Two additional index variants were in perfect linkage disequilibrium (LD) ( $r^2=1.00$ ) with protein-altering variants in *KLKB1* and *LMAN1*, as described in the section on fine-mapping below.

### Fine-mapping for causal variants in the cTnI loci

We identified 14 credible sets (Supplementary Material, Table S9) of genetic variants in the cTnI loci using SuSiE. For each credible set, there was a 95% cumulative posterior probability that it contained at least one causal variant. The sizes of the credible sets ranged from 1 variant to 64 variants (in the HLA region and one region on chromosome 10), providing varying evidence for the importance of specific variants. Nine of the cTnI index variants (rs7650482, rs12331618, rs7080536, rs7481951, rs4283165, rs8024538, rs1801690, rs151313792 and rs12604424)

were found in a credible set, and one of these credible sets only contained the index variant itself (rs7080536), suggesting that this was the most likely causal variant in the locus. The two loci with the lowest  $P\text{-values}$  (chromosomes 10 and 11) gave rise to two credible sets each, indicating multiple independent signals. However, the credible sets were in close proximity or overlapping, and therefore, the seemingly independent signals might have resulted from uncaptured LD structures.

Annotation of the variants in each credible set (Supplementary Material, Table S10) revealed four additional protein-altering variants in moderate-to-high LD with non-exonic index variants (Table 1): rs11718898 in *CAND2*, correlation  $r^2$  in HUNT ( $r^2_{\text{HUNT}}=0.91$  with index variant rs7650482 (Fig. 1A); rs3733402 in *KLKB1*,  $r^2_{\text{HUNT}}=1.00$  with index variant rs12331618 (Fig. 1B); rs1132339 in *TNFAIP2*,  $r^2_{\text{HUNT}}=0.65$  with index variant rs4283165 (Fig. 1C); rs2298711 in *LMAN1*,  $r^2_{\text{HUNT}}=1.00$  with index variant rs12604424 (Fig. 1D). The rs2298711 protein-altering variant was reported as index variant in the previous GWAS in GS:SFHS (13). Furthermore, we found a rare exonic frameshift substitution (rs137854521 in *ANO5*,  $r^2_{\text{HUNT}}=0.003$ ,  $D'=1.00$  with the more common index variant rs7481951) in the second credible set in the same region as rs7481951, and a variant in another credible set was located in the *F12* 5' untranslated region (UTR).

### Prioritization of genes, pathways and tissues

To further explore the underlying biology of the cTnI-associated loci, we used Data-driven Expression Prioritized Integration for Complex Traits (DEPICT) to identify candidate causal genes at each locus. After removing results with a false discovery rate (FDR) above 5%, DEPICT prioritized 10 genes from suggestive ( $P\text{-value} < 1 \times 10^{-5}$ ) loci based on shared predicted functions with genes from the other associated loci: *NRAP*, *ALPK3*, *SYNPO2L*, *TRIM63*, *CHODL*, *CAND2*, *LSMEM2*, *AOPEP*, *FHOD3* and *VCL* (Supplementary Material, Table S6). Most of these (*NRAP*, *ALPK3*, *SYNPO2L*, *CAND2*, *VCL*, *FHOD3*) were in the 12 cTnI loci, but not always the nearest gene to the index variant. No gene sets or tissue types were significantly enriched ( $FDR < 5\%$ ) with genes or gene expression from cTnI loci. However, the five tissues most enriched with expression of genes from suggestive cTnI loci were heart, heart ventricles, muscles, heart atria and atrial appendages (Supplementary Material, Table S7). Among 14 461 reconstituted gene sets, the sets 'costamere', 'abnormal myocardial fiber morphology', 'muscle organ development', 'muscle structure development' and 'DAG1 PPI subnetwork' were the five most enriched with genes from suggestive cTnI loci (Supplementary Material, Table S8).

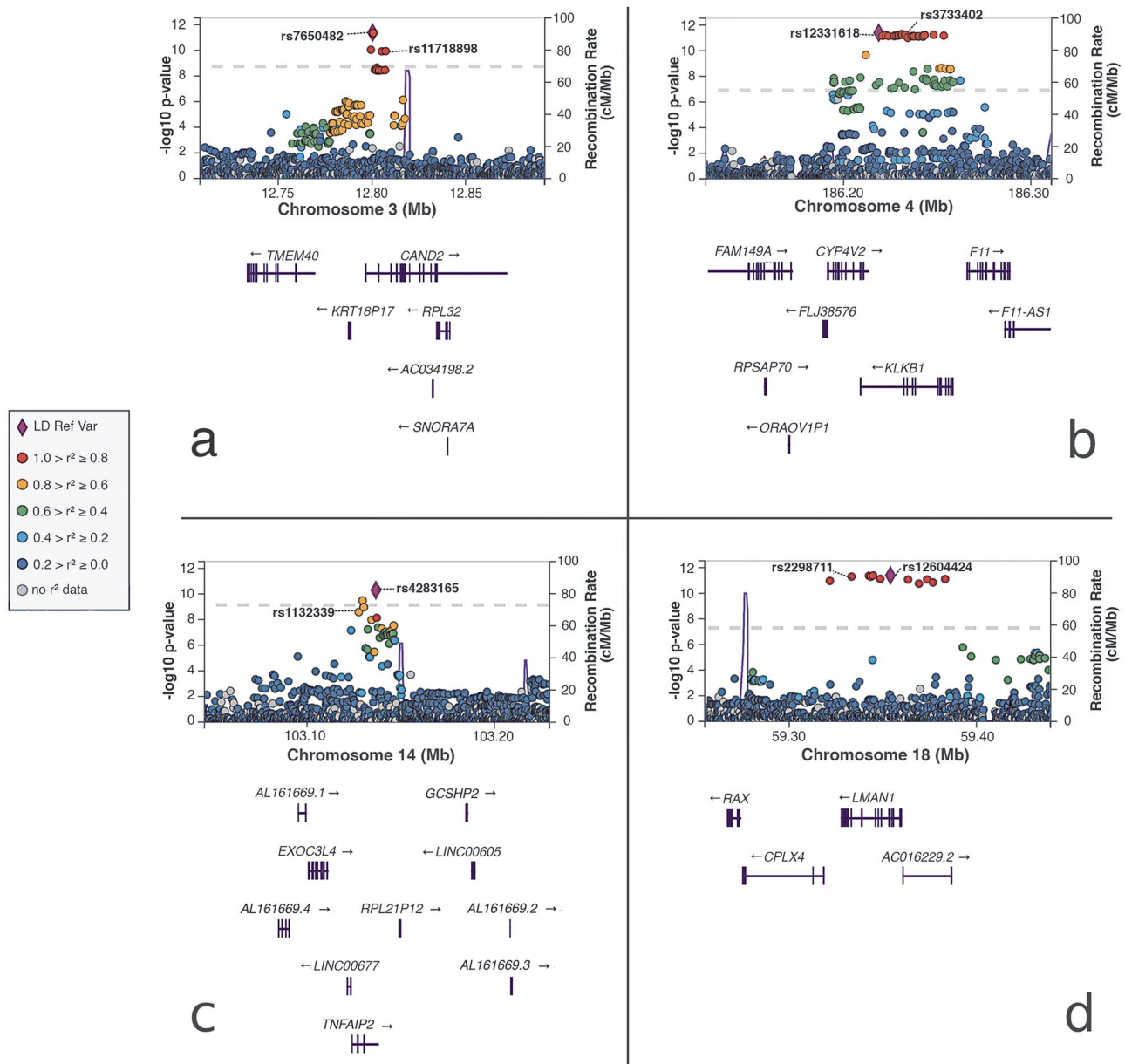
### Heritability of cTnI concentration

We estimated the proportion of phenotypic variance explained by 358 956 genotyped autosomal variants using genome-wide complex trait analysis (GCTA) and found a narrow-sense single-nucleotide variant (SNV) heritability of cTnI concentration in HUNT (variance explained,  $V_g/V_p \pm 1$  SE) to be  $0.15 \pm 0.01$ ,  $P\text{-value} 2.63 \times 10^{-32}$ . The HUNT cohort has a high degree of relatedness and is therefore more genetically homogeneous than cohorts with fewer relatives. Including more rare variants from the TOPMed panel would further increase the homogeneity of the dataset. This might, in part, explain the lower heritability observed in HUNT than in previous studies (13,17).

Table 1. Index variants in genome-wide significant cTnI loci

Novelty	rsID	Chr:Pos:Ref:Alt	Consequence	Reference Gene	AF	Effect Size	SE	P-value
Novel	rs7650482	3:12800305:A:G	intronic, $r^2 = 0.91$ with protein-altering variant	CAND2	0.64	0.04	0.01	$4 \times 10^{-10}$
Known	rs12331618	4:186218785:A:G	intergenic; $r^2 = 1.00$ with protein-altering variant	CYP4V2,KLKB1	0.52	0.04	0.01	$2 \times 10^{-12}$
Novel	rs2731672	5:177415473:T:C	intergenic	F12,GRK6	0.74	0.05	0.01	$9 \times 10^{-14}$
Novel	rs9391746	6:32715903:G:T	intergenic	HLA-DQB1; HLA-DQA2	0.07	0.08	0.01	$1 \times 10^{-8}$
Known	rs2270552	10:74103992:C:T	intronic	VCL	0.68	-0.06	0.01	$3 \times 10^{-19}$
Novel	rs7080536	10:113588287:G:A	protein-altering	HABP2	0.04	-0.10	0.01	$1 \times 10^{-12}$
Known	rs7481951	11:22250324:A:T	protein-altering	ANO5	0.59	0.05	0.01	$2 \times 10^{-19}$
Novel	rs4283165	14:103137158:G:A	3' UTR, $r^2 = 0.65$ with protein-altering variant	TNFAIP2	0.65	0.04	0.01	$7 \times 10^{-9}$
Novel	rs8024538	15:84825188:A:T	intronic	ALPK3	0.53	0.04	0.01	$7 \times 10^{-9}$
Novel	rs1801690	17:66212167:C:G	protein-altering	APOH	0.06	0.10	0.01	$1 \times 10^{-14}$
Novel	rs151313792	18:36681510:G:A	protein-altering	FHD3	0.004	0.26	0.05	$1 \times 10^{-8}$
Known	rs12604424	18:59353974:T:C	Intronic, $r^2 = 1.00$ with protein-altering variant	LMAN1	0.13	0.06	0.01	$1 \times 10^{-11}$

The variants are given with chromosome (Chr), genomic position in GRCh38 (Pos), reference (Ref) and alternate (Alt) alleles. Consequence, allele frequency (AF) and effect size (1 standard deviation of the inverse rank transformed cTnI concentration) with standard error (SE) are given for the alternate allele.



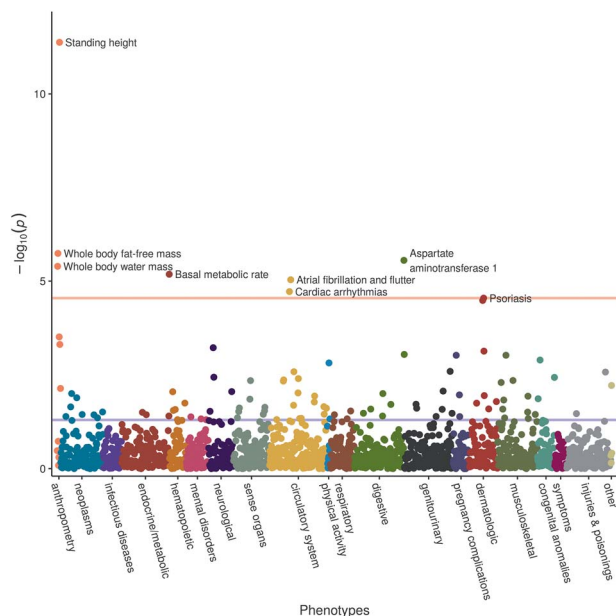
**Figure 1.** LocusZoom plots of cTnI loci with protein-altering variants in moderate or high LD with the index variant: Chromosome positions (GRCh38) are given on the x-axes, and the meta-analysis  $-\log_{10}(P\text{-value})$  for the variants is given on the y-axis. The genome-wide significance  $P\text{-value}$  threshold ( $5 \times 10^{-8}$ ) is indicated by the dotted line. The correlation  $r^2$  for each variant is indicated by colors relative to the index variants (LD Ref Var) in each panel: (A) The CAND2 locus on chromosome 3 with index variant rs7650482. A protein-altering variant in CAND2, rs11718898 (p.Val77Ala), is in high LD with the index variant. (B) The KLKB1 locus on chromosome 4 with index variant rs12331618. A protein-altering variant in KLKB1, rs3733402 (p.Ser143Asn, p.Ser105Asn), is in perfect LD with the index variant. (C) The TNFAIP2 locus on chromosome 14 with index variant rs4283165. A protein-altering variant in TNFAIP2, rs1132339 (p.Gln282Glu), is in moderate LD with the index variant. (D) The LMAN1 locus on chromosome 18 with index variant rs12604424. A protein-altering variant in LMAN1, rs2298711 (p.Met410Leu), is in perfect LD with the index variant.

### Variants associated with tissue-specific gene expression

Five of the cTnI index variants were associated ( $P\text{-value} < 10^{-9}$ ) with the expression of one or more genes in at least one tissue type (Supplementary Material, Table S11). Two of these variant-gene associations were found in the cardiovascular system: rs7481951 was associated with the expression of ANO5 in aortic and tibial arteries and rs12331618 was associated with the expression of F11 in tibial arteries.

To discover shared causal variants for cTnI concentration and expression levels of nearby genes in different tissues, we used

the cis-expression quantitative trait locus (eQTL) data from the Genotype-Tissue Expression (GTEx) portal v8 and performed a Bayesian colocalization analysis of the gene expression signals and cTnI concentration signals. Four cTnI loci were colocalized (posterior probability of common causal variant  $>75\%$ ) with the cis-eQTL signals for a gene in at least one tissue (Supplementary Material, Table S12): The expression of coagulation factor F11 in tibial arteries (rs12331618), the expression of coagulation factor F12 in liver (rs2731672), the expression of BMS1P4 (pseudogene) in whole blood (rs2270552), the expression of ADK in the sigmoid colon (rs2270552), as well as the expression of lncRNA



**Figure 2.** GRS-PheWAS: associations of GRS (increasing cTnI concentrations) with 1688 phecodes and 83 continuous traits and biomarkers in UK Biobank.

gene *Lnc-WDR73-10* in the left heart ventricle, mammary breast tissue and brain frontal cortex (rs8024538). The association signal for *ADK* was also near the posterior probability cut-off of 75% in aortic tissue (rs2270552) (Supplementary Material, Table S12).

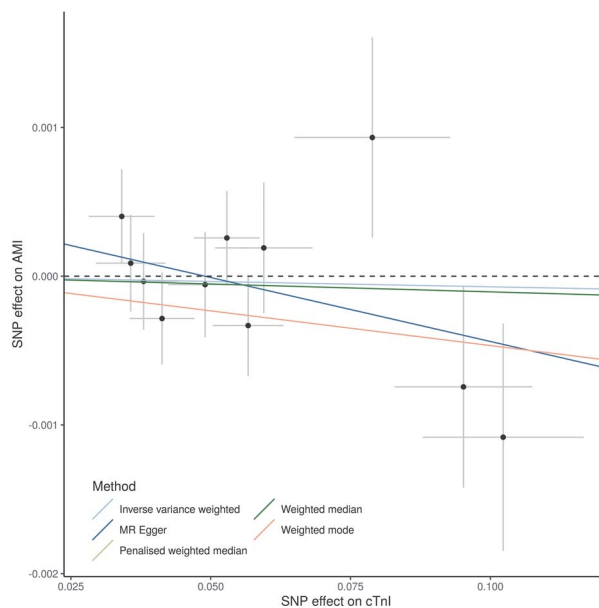
### Phenome-wide association study of cTnI loci and cTnI genetic risk score

In a phenome-wide association study (PheWAS), four of the cTnI index variants were associated ( $P$ -value  $< 2.8 \times 10^{-5}$ ) with diseases or continuous traits in UK Biobank (Supplementary Material, Tables S13 and S14) after correction for multiple testing: The variant rs7650482 was associated with cardiac arrhythmias, in particular atrial fibrillation and flutter, as well as waist hip ratio and standing height. In addition, the variant rs12331618 was associated with pulmonary heart disease and phlebitis/thrombophlebitis, the variant rs2731672 was associated with standing height and basal metabolic rate and the variant rs9391746 was mainly associated with autoimmune inflammatory conditions.

We further identified seven phecodes and continuous traits in UK Biobank that were associated with the genetic risk score (GRS) based on the cTnI index variants ( $n=12$ , variance explained = 1.2%) after correction for multiple testing (Fig. 2; Supplementary Material, Tables S15 and S16): cardiac arrhythmias in general, and the sub-phenotype atrial fibrillation and flutter in particular, as well as standing height, aspartate aminotransferase 1, whole body fat free mass, whole body water mass and basal metabolic rate.

### Mendelian randomization

To investigate the causal effect of circulating cTnI on both AMI and HF, we performed two-sample MR using 11 of 12 index variants from our meta-analysis as an instrument for cTnI [F-statistic = 44,  $r^2 = 0.012$  in the independent sample ( $N = 3424$ )], and summary-level data on AMI (5948 AMI cases, 355 246 controls from UK Biobank) and HF (47 309 cases and 930 014 controls



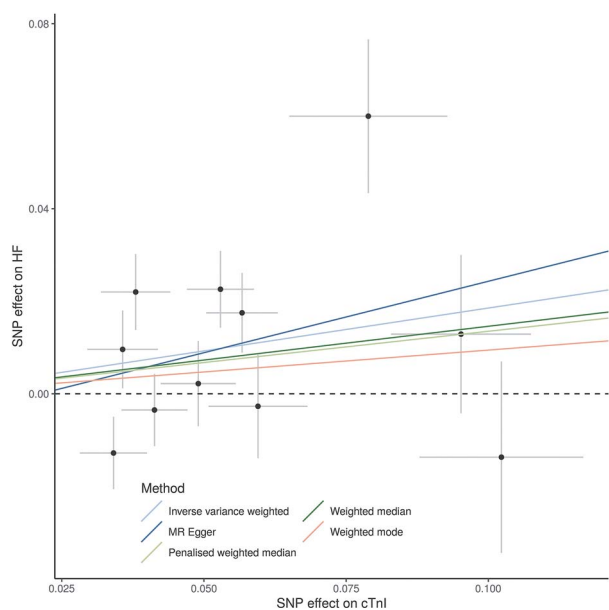
**Figure 3.** Mendelian randomization analysis of the causal effect of cTnI on AMI: The analysis was based on summary statistics from the meta-analysis of HUNT and GS:SFHS ( $n=48\,115$ ) for cTnI and from a GWAS of AMI in UK Biobank from the Neale laboratory (cases/controls = 59 948/355 246). Effect allele–cTnI associations given on x-axis and effect allele–AMI associations given on y-axis. All associations are given with 1 standard deviation error bars.

from the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) Consortium].

There was no evidence of a causal effect of circulating cTnI on AMI (inverse variance-weighted (IVW) odds ratio (OR) = 1.00, 95% confidence interval (CI) [0.99, 1.00]) (Supplementary Material, Table S17, Fig. 3). Bidirectional MR however suggested evidence for a potential causal effect of cTnI on HF (IVW OR = 1.20, 95% CI: [1.00, 1.45]) in addition to the well-known effect of HF on cTnI concentration (IVW effect size = 0.14, 95% CI: [0.00, 0.28]) (Supplementary Material, Table S17). Sensitivity analyses showed similar results between all methods (weighted mode, weighted median, penalized weighted median, MR-Egger), although wider confidence intervals due to low power, and Steiger filtering did not change the results (Supplementary Material, Table S17; Fig. 4). The MR-Egger intercept was close to zero for both outcomes, giving no strong evidence of direct effects of the index variants on AMI or HF that are not mediated by cTnI (horizontal pleiotropy). However, there was some evidence for heterogeneity in the analysis of HF (heterogeneity statistic  $Q=27$ ,  $P$ -value = 0.003), but not for the analysis of AMI ( $Q=9$ ,  $P$ -value = 0.50). The heterogeneity in the HF analysis could possibly reflect horizontal pleiotropy, which would violate the instrumental variable analysis assumptions or indicate multiple underlying mechanisms. Because HF development is commonly preceded by increase in left ventricular mass, we further investigated the association of cTnI on HF. We found a weak association of circulating cTnI on left ventricular mass (IVW effect size = 0.07, 95% CI:  $-0.11, 0.27$ ), but the effect was attenuated and changed direction in sensitivity analyses (Supplementary Material, Table S17).

### Discussion

Our GWAS meta-analysis of 48 115 individuals in HUNT and GS:SFHS revealed a genetic contribution to circulating protein



**Figure 4.** Mendelian randomization analysis of the causal effect of cTnI on HF. The analysis was based on summary statistics from the meta-analysis of HUNT and GS:SFHS ( $n = 48\,115$ ) for cTnI and from a meta-analysis of HF in the HERMES Consortium (cases/controls = 47 309/930 014). Effect allele–cTnI associations given on x-axis and effect allele–HF associations given on y-axis. All associations are given with 1 standard deviation error bars. The outlier variant with a large effect size for both traits is rs9391746 from the HLA region.

concentrations of cTnI with associations to several biological processes. We identified 12 loci consistently associated with cTnI concentrations across both studies, confirming four of the five previously unreplicated loci (13). Furthermore, we suggest several novel loci for change in cTnI with time, confirm cTnI as a non-causal biomarker for AMI and observe indications that cTnI may have a causal effect on HF development.

Cardiac troponin I and T are important biomarkers for cardiovascular disease and especially used to diagnose AMI in patients with symptoms of acute myocardial ischemia (1,18). Moreover, cTnI and cTnT have been found to provide strong and independent information regarding future risk for cardiac morbidity and mortality among subjects of the general population (19). Of note, while cardiac troponins are excellent markers of AMI in the acute setting, high-sensitivity troponins seem more related to incident HF events than to future AMI in the general population (20,21). Hence, data from several independent cohorts support a model of cardiac troponins as either directly or indirectly associated with HF development. This model is supported by high-sensitivity troponin concentrations being correlated with indices of structural heart disease and most prominently left ventricular mass (20,22–24).

Using genetic data from two large cohorts from the general population (HUNT and GS:SFHS), we now provide new and additional information related to a role for cardiac troponins in cardiovascular disease. Four protein-altering index variants have previously been associated with cardiovascular health or with muscle development and injury: Firstly, a protein-altering variant in the fibrinolysis-related gene *HABP2* (rs7080536) that reduces the activity of the translated protein (25), coagulation factor VII activating protease, and has been associated with carotid stenosis (26) and venous thromboembolism (27). Secondly, a protein-altering variant in *APOH* (rs1801690) that has

been associated with the concentrations of creatine kinase (28), which is an unspecific biomarker for acute muscle injury, and with activated partial thromboplastin time (28), a measure of coagulation. Third, a previously reported protein-altering variant in *ANO5* (rs7481951) that has been suggested to be causal for adult-onset muscular dystrophy (29). Finally, we identified a rare protein-altering variant (rs151313792) in *FHOD3*, a gene that regulates actin assembly in cardiac sarcomeres (30) and has been associated with dilated cardiomyopathy (31) and left ventricular ejection fraction (32).

The remaining index variants were in close proximity to genes previously associated with the cardiovascular system, either through disease associations or links to cardiovascular development, structure and function: *ALPK3*, *FHOD3*, *VCL* and *CAND2* have been implicated in heart development and/or function (28,33–35), and *TNFAIP2* is central in blood vessel formation (36). Both *ALPK3* and *VCL* are associated with cardiomyopathy (4,31,33), and *CAND2* is associated with atrial fibrillation (37) and also creatine kinase concentrations (28). Furthermore, the *KLKB1/CYP4V2* locus has been associated with atherosclerosis (38), and the *KLKB1* and *F12* genes are both involved in several proteolytic reaction cascades in the cardiovascular system (39). These cascades are associated with coagulation/fibrinolysis, blood pressure regulation and inflammation (39). *LMAN1* has also been associated with coagulation (40), and both *TNFAIP2* and *HLA-DQB1* are associated with vascular inflammation (36,41). Both coagulation and atherosclerosis are pathobiological components crucial to the progress of acute exacerbations of coronary artery disease, and blood pressure regulation will impact left ventricular mass, and this is therefore comparable with previous data on cardiac troponins and left ventricular structure and function. The *F12/GRK6* locus has additionally been associated with chronic kidney disease (42). Several of the protein-altering and regulatory region variants that we identified had previously also been linked to some of the above-mentioned processes (25–29,38,43,44). Excluding individuals with impaired kidney function or with a history of diabetes, atrial fibrillation and flutter, cardiomyopathy or HF, did not attenuate the effect sizes of any of the 12 index variants. This supported that the genetic associations with cTnI concentrations were not driven by these clinical endpoints.

Fine-mapping of the cTnI-associated loci pointed to additional protein-altering and regulatory region variants and further indicated two separate causal signals in each of the two most significant cTnI loci. A rare *ANO5* frameshift substitution (rs137854521) was possibly driving the second independent signal in the most significant locus as indicated by the fine-mapping analysis. A variant in another credible set was in the *F12* 5' UTR and is associated with coagulation factor XII deficiency (rs1801020,  $r^2_{\text{HUNT}} = 0.99$  with rs2731672). The protein-altering variant in *HABP2* was alone in its credible set, providing additional support for its contribution to circulating cTnI concentrations in the general population. On the other hand, a large credible set from the barely statistically significant HLA region contained only intergenic variants, none of which were the index variant, weakening the evidence for a role of this specific variant in troponin biology. This is not surprising, given that variants in this region are known to be difficult to impute and interpret (45).

With a small number of cTnI loci, the DEPICT analysis had limited power to detect enrichment of genes in gene sets or gene expression in tissue types. Despite this limitation and a  $\text{FDR} > 5\%$ , we found that the expression of genes in cTnI loci with  $P\text{-value} < 10^{-5}$  was most enriched in the heart, which is as expected given that cTnI is only expressed in cardiac

myocytes, and the basis for the excellent performance of cardiac troponins as cardiovascular biomarkers (46). Furthermore, the gene sets most enriched with cTnI locus genes were 'costamere' and 'abnormal myocardial fiber morphology'. The costamere is a structure that is important for (cardio)myocyte force transmission and plays a significant role in sarcomeric remodeling (47). Both gene sets link the cTnI loci to cardiac structure. Finally, DEPICT prioritized several genes in the cTnI loci that were associated with heart development, structure and function, and with cardiovascular diseases: NRAP, which is located in the HAP2 locus, is involved in sarcomere assembly during cardiomyocyte development (48) and is associated with familial cardiomyopathies (49), and mutations in TRIM63 have been associated with left-ventricular dysfunction and hypertrophic cardiomyopathy (50). Likewise, SYNPO2L, which is located in the second most significant locus on chromosome 10, but further away from the index variant than VCL, is involved in sarcomeric signal transduction (51) and cardiac development (52). The associations with sarcomere structure and function are plausible based on the biology as cardiac troponins are essential components of the sarcomere.

While we identified protein-altering variants in more than half of the loci, several statistically significant variants were located outside of the coding regions. Non-coding variants might however have regulatory functions, and we therefore investigated their associations with variation in gene expression across 49 tissues. Four cTnI loci with non-coding index variants were highly colocalized with the expression signals for a nearby gene in at least one tissue, including the expression of coagulation factors in arteries and liver, and a protein degradation activator in blood. The colocalization with expression signals for adenosine kinase was also quite strong in the aorta. This protein has been implicated in cardiomyocyte microtubule dynamics and cardiac adaptation to high blood pressure and is found to attenuate cardiac hypertrophy (53). These findings corroborate the role of these loci in cTnI biology, possibly in relation to the response to cardiac injury and myocardial stress and to the breakdown of circulating cTnI.

The single-variant genome-wide association tests in the UK Biobank indicated some level of pleiotropy of cTnI variants: Seven of 12 index variants were associated with other traits than cTnI. These traits included cardiovascular diseases, waist-hip ratio, standing height, aspartate aminotransferase 1 concentrations and basal metabolic rate. Waist-hip ratio has been linked to future risk of cardiovascular outcomes (54), standing height correlates with blood pressure, cardiac output and vascular resistance (55) and the basal metabolic rate could potentially affect cTnI degradation. Aspartate aminotransferase 1 is an enzyme released from the heart in patients with AMI (56) but is also related to amino acid metabolism in the liver and is used as an unspecific biomarker for liver cell damage (57). This enzyme could thus both be related to cardiovascular disease and possibly to degradation and clearance of circulating cTnI in the liver. However, there were no associations between cTnI variants and the more liver-specific enzyme alanine aminotransferase 1, which could indicate that the association is related to the cardiovascular system, rather than to liver damage. Hence, the mechanism whereby aspartate aminotransferase 1 influences circulating cTnI concentrations will require additional studies. The identified HLA region variant in our study was mainly associated with autoimmune and inflammatory diseases, and this is relevant for cardiovascular diseases (58), but more studies are needed, especially since interpretation of specific HLA region variants is difficult.

The GRS combining the 12 cTnI index variants explained 1.2% of variation in cTnI concentrations in an independent sample from HUNT. In UK Biobank, it was associated with some of the same traits as the single variants, which is not surprising: standing height, basal metabolic rate, aspartate aminotransferase 1 concentrations and cardiac arrhythmias, including atrial fibrillation and flutter. Furthermore, the GRS was associated with whole-body fat-free mass and whole-body water mass, which was not seen for any single variant. By performing two-sample MR, we confirmed a non-causal role of cTnI in AMI. In contrast, we found indications of a potential causal effect of cTnI in HF development, although the estimate was imprecise. This was surprising given the current opinion of cTnI as a passive biomarker of cardiac injury (46), but in line with some animal studies suggesting that circulating cTnI can trigger immune responses causing HF (10,11). Follow-up analyses of left ventricular mass, which is known to increase during HF development, however, did not support this finding.

In conclusion, our study highlights diverse genetic determinants of cTnI concentration in the general population and corroborates cTnI as a non-causal biomarker for AMI and HF development in the general population. Furthermore, it suggests several novel loci for change in cTnI over time, although future studies are required to validate these findings and to explore possible mechanisms.

## Materials and Methods

### Cohort descriptions

The recruitment and design of the HUNT and GS:SFHS studies have been reported elsewhere (15,59). Participants (aged  $\geq 20$  years in HUNT and 35–65 years in GS:SFHS) have been genotyped using Illumina HumanCoreExome array 12 v.1.0 or v.1.1 in HUNT and Illumina HumanOmniExpressExome-8 v1\_A or v1-2 in GS:SFHS. Sample and variant quality control was performed using standard practices and has been reported in detail for both studies elsewhere (60,61). All variants were imputed from the TOPMed reference panel(16) (freeze 5) using Minimac4 v1.0 (<https://genome.sph.umich.edu/wiki/Minimac4>). The reference panel is composed of 53 831 multi-ethnic samples and 308 107 085 SNVs and indel variants at high depth (mean read depth 38.2X). The individuals included in the analyses were of European ancestry.

### Cardiac troponin I

High-sensitivity cTnI concentrations (Architect STAT, Abbott Laboratories, Abbott Diagnostics, USA.) were measured from serum samples in both studies using the manufacturers' protocol and quality assurance scheme.

In HUNT, non-fasting serum samples were drawn in 1995–1997 (HUNT2), 2006–2008 (HUNT3) and 2017–2019 (HUNT4). The HUNT2 and HUNT3 samples were centrifuged at room temperature and stored at  $-80^{\circ}\text{C}$  after collection. The HUNT2 samples were thawed and refrozen once in 2008 and subsequently stored at  $-20^{\circ}\text{C}$ . High-sensitivity cTnI concentrations were analyzed in 2014 from HUNT2, in 2015 from HUNT3 and were analyzed consecutively in 2017–2019 from fresh serum samples in HUNT4. We combined cTnI measurements from all studies into one variable for cTnI concentration: If an individual had measurements from multiple HUNT surveys, we used the earliest measurement in the analyses, assuming participants to be less exposed to potential confounding factors at a younger age. After prioritization,



29 839 individuals of European ancestry had measurements of cTnI in HUNT, of which 23 990 were above or equal to the lower limit of detection (1.2 ng/l) reported for the assay (62). Measurements below 1.2 ng/l are imprecise on an individual level but were included since they collectively could add information to the GWAS analysis.

We defined change in cTnI concentration per time as the difference in cTnI concentration from HUNT2 to HUNT3 divided by the follow-up time. The median follow-up time was 10 years and 7 months. In total, 5178 participants had data of change in cTnI over time.

In GS:SFHS, fasting blood samples were collected between 2006 and 2010. Serum samples were centrifuged, and serum aliquots stored at  $-80^{\circ}\text{C}$  until future biochemical analyses. A total of 18 276 individuals had measurements of cTnI in GS:SFHS. Measurements below the reported limit of detection (1.2 ng/l) (62) were reported as half of the limit of detection (0.6 ng/l) since the original measurements were unavailable.

### Association analyses

We used a linear mixed model regression under an additive genetic model for each variant as implemented in BOLT-LMM (v.2.3.4) (63) to control for relatedness between the samples in all analyses.

We included age, sex, genotyping batch and the first 10 genetic principal components (PCs) of ancestry as covariates in both HUNT and GS:SFHS. Additionally, we included as a covariate in HUNT the survey from which the serum sample was collected for cTnI measurement. We applied rank-based inverse normal transformation for the cTnI concentration after adjusting for age and sex using linear regression (additionally adjusting for survey in HUNT). Variants with a minor allele count  $<10$  or imputation  $R^2 < 0.3$  were excluded, leaving 21 233 433 variants in the HUNT analysis and 21 677 569 variants in the GS:SFHS analysis. We performed genomic control correction after each analysis.

### Meta-analysis

We performed a fixed-effect IVW meta-analysis of 26 711 992 variants imputed in up to 48 115 individuals using METAL (64). We considered genetic loci reaching a  $P$ -value  $< 5 \times 10^{-8}$  in the meta-analysis for follow-up analysis.

### Sensitivity analyses

To investigate if the identified genetic loci could be explained by clinical endpoints associated with chronically elevated cardiac troponin levels, we performed a sensitivity analysis of cTnI concentration in HUNT, excluding 5253 individuals with a current or known history of comorbidities [atrial fibrillation (ICD9 427.3, ICD10 I48), diabetes mellitus (self-reported), HF (ICD9 428, ICD10 I50), cardiomyopathy (ICD9 425, ICD10 I42) or impaired kidney function (estimated glomerular filtration rate  $< 60$  ml/min/1.73 m<sup>2</sup>) in the HUNT survey where the serum sample was collected for cTnI measurement].

### Heritability estimation

We estimated the narrow-sense (additive) SNV heritability of cTnI concentration in HUNT using GCTA (65,66). We created a genetic relationship matrix (GRM) based on 358 956 genotyped autosomal variants in the 29 839 HUNT participants with

troponin measurements and used the GRM with GCTA-GREML (genomic relatedness-based restricted maximum-likelihood) to estimate the variance in cTnI concentration explained by the variants. Age, sex, genotyping batch and HUNT survey were used as covariates in the analysis, and the cardiac troponin measurement was transformed to normality with rank-based inverse normal transformation after regression on age, sex and HUNT survey prior to analysis.

### Variant annotation and fine-mapping

We annotated genetic variants using ANNOVAR (v.2019Oct24) (67). For fine-mapping of the cTnI loci, we applied SuSiE (v0.7.1) (68), which uses iterative Bayesian stepwise selection to estimate the number of causal variants in each locus and create credible sets with a 95% cumulative posterior probability for containing a causal variant. The LD matrix used with SuSiE was calculated from the imputed variants of 5000 unrelated individuals in HUNT, and variants only imputed in GS:SFHS were therefore excluded from the fine-mapping. We visualized the fine-mapped regions with LocusZoom (69).

### Tissue, gene and gene set enrichment tests

We performed gene set enrichment, gene prioritization and tissue/cell type enrichment tests on the cTnI loci using DEPICT (v1.1, release 194) (70) with default parameters. We used LiftOver from the UCSC human genome browser (71) to convert the genomic positions of the cTnI loci from GRCh38 to GRCh37 prior to the analysis and analyzed two sets of independent variants meeting two different  $P$ -value cut-offs ( $P$ -value  $< 1 \times 10^{-5}$  and  $P$ -value  $< 5 \times 10^{-8}$ ). The HLA region was excluded from the analyses.

### Expression quantitative trait locus tests

To identify cTnI index variants associated with the expression level of a gene in specific tissue types, we downloaded single-tissue cis-eQTL data from the GTEx portal, data set v8 (<https://www.gtexportal.org>). We identified all statistically significant associations [ $P$ -value  $< 1 \times 10^{-9}$  ( $5 \times 10^{-8}/49$  tissues)] between cTnI index variants and gene expression levels in any tissue type. To further assess if any cis-eQTL signals and GWAS meta-analysis signals were consistent with a shared causal variant for cTnI concentration and gene expression, we used Bayesian colocalization analysis as implemented in the R package coloc (72). For each tissue type, we analyzed all genes whose expression was associated ( $P$ -value  $< 5 \times 10^{-8}$ ) with at least one cTnI-associated variant ( $P$ -value  $< 5 \times 10^{-8}$ ), using effect sizes and standard errors for each variant-trait association as input. The coloc software estimated the variance in each trait (cTnI concentration or gene expression level) from the sample sizes and minor allele frequencies. We set the prior probability of a genetic variant being associated with only cTnI concentrations, only gene expression or both traits to be  $10^{-4}$ ,  $10^{-4}$  and  $10^{-6}$ , respectively. We considered posterior probabilities (PP4) above 75% to give strong support for a common causal variant for the two traits in a given tissue, posterior probability between 50 and 75% to be inconclusive or to give weak support for a common causal variant and posterior probabilities  $< 50\%$  to indicate no support for a common causal variant between the two traits.

## GRS for cTnI

We used R (v3.6.2) (73) to construct a GRS for cTnI, by summing the product of the effect size and the estimated allele count for the 12 index variants in genome-wide significant loci ( $P$ -values  $< 5 \times 10^{-8}$ ). To test the association between the GRS and cTnI in an independent sample, we performed the GWAS on cTnI concentrations in 26 415 HUNT participants, excluding 3424 individuals who were unrelated (no 3rd degree relatives or closer) both to each other and to the individuals in the GWAS. We used KING (v2.1.5) (74) to estimate the relatedness. The smaller GWAS in HUNT ( $N=26\,415$ ) was meta-analyzed with the full GWAS from GS:SFHS to obtain effect estimates independent of the excluded individuals. We constructed the GRS from the effect size estimates from this meta-analysis and used a linear regression model to test the association between the GRS and serum concentrations of cTnI (inverse rank transformed to normality) in the independent sample of 3424 unrelated HUNT participants.

## PheWAS tests

We tested the association of the GRS (GRS-PheWAS), as well as the individual index variants (single variant PheWAS), with 1688 phenotype codes ('phecodes') defined by aggregating related ICD codes (75), and 83 continuous biomarkers and traits in participants of white British ancestry in UK Biobank. We calculated a GRS for each participant as described in the above paragraph, using TOPMed imputed estimated allele counts and effect sizes from the meta-analysis. We used a logistic or linear regression model respectively to assess the association of the single variant estimated allele counts or inverse normalized GRS and each phecode or continuous biomarker. For the GRS-PheWAS, we included as covariates sex and birth year for binary traits, and sex and age at measurement for continuous traits. For the single-variant PheWAS, we used GWAS summary statistics generated with SAIGE (v.29.4.2) (76), with sex and the first four PCs as covariates in addition to age at initial assessment for quantitative traits and birth year for binary traits. To correct for multiple testing of phecodes and continuous variables, we used a Bonferroni-corrected significance cut-off of  $2.8 \times 10^{-5}$ .

## MR of cTnI on AMI and HF

To assess causal associations of circulating cTnI on AMI or HF, we performed IVW two-sample MR analyses using R (v3.6.2) (73) with the TwoSampleMR (77) and MRInstruments (78) libraries. We used the effect sizes of 11 index variants from the meta-analysis as genetic instrument for cTnI, while we found the respective associations and effect sizes of the instrument variants for AMI (ICD10 I21) and HF (ICD10 I50) from independent genome-wide summary level data [5948 AMI cases versus 355 246 controls from UK Biobank (79) available from the Neale laboratory (<http://www.nealelab.is/ukbiobank>) and 47 309 HF cases versus 930 014 controls from the HERMES Consortium (52) available from the Cardiovascular Disease Knowledge Portal (<http://www.broadcvdi.org>)]. The rare cTnI index variant rs151313792 was excluded from both analyses because it was not present in either outcome data set, and we were unable to identify a proxy variant. In the analysis of HF, the variant rs8039472 was used as a proxy for rs8024538 ( $r^2_{\text{HUNT}}=0.98$ ) because rs8024538 was not present in the HF data. We performed sensitivity analyses using Steiger filtered IVW, weighted mode, weighted median, penalized weighted median and MR Egger. To evaluate the reverse causal association of

HF on cTnI concentration, we also performed an IVW two-sample MR analysis using the effect sizes of 11 index variants for HF, and the respective associations and effect sizes of the same variants from our GWAS meta-analysis of cTnI. We further performed sensitivity analyses using left ventricular mass, which is associated with HF development. We found the associations and effect sizes of these variants for left ventricular mass from an independent GWAS in 18 257 participants in UK Biobank (80).

## Ethics

Participation in HUNT is based on informed consent, and the study has been approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics in Central Norway (REK Reference number: 2015/2294).

The GS:SFHS obtained written informed consent from all participants and received ethical approval from the National Health Service Tayside Committee on Medical Research Ethics (REC Reference number: 05/S1401/89).

This study was covered by the ethics approval for UK Biobank studies (application 24460) from the NHS National Research Ethics Service on June 17, 2011 (Ref 11/NW/0382) and extended on May 10, 2016 (Ref 16/NW/0274).

## Supplementary Material

Supplementary Material is available at HMG online.

## Data availability

Data generated or analyzed during this study are available from the corresponding authors upon reasonable request.

## Acknowledgements

The Trøndelag Health Study (The HUNT Study) is a collaboration between HUNT Research Center (Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology), Trøndelag County Council, Central Norway Regional Health Authority and the Norwegian Institute of Public Health.

*Conflict of Interest statement.* T.O. reports research support and honoraria from Abbott Diagnostics and Roche Diagnostics and honoraria from Siemens Healthineers, manufacturers of high-sensitivity cardiac troponin assays. The other authors declare no competing interests.

## Funding

The genotyping in HUNT was financed by the National Institutes of Health (grant number NIH R35 HL135824-03); University of Michigan; the Research Council of Norway; the Liaison Committee for Education, Research and Innovation in Central Norway; and the Joint Research Committee between St Olav's Hospital and the Faculty of Medicine and Health Sciences, NTNU. The K.G. Jepsen Center for Genetic Epidemiology is funded by Stiftelsen Kristian Gerhard Jepsen (grant number SKGJ-MED-015); Faculty of Medicine and Health Sciences, NTNU; The Liaison Committee for education, research and innovation in Central Norway; and the Joint Research Committee between St. Olavs Hospital and the Faculty of Medicine and Health Sciences, NTNU. The cardiac

troponin I analyses in HUNT were sponsored by Abbott Diagnostics. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

## References

- Thygesen, K., Alpert, J.S., Jaffe, A.S., Chaitman, B.R., Bax, J.J., Morrow, D.A., White, H.D., Corbett, S., Chettibi, M., Hayrapetyan, H. et al. (2018) Fourth universal definition of myocardial infarction (2018). *138*, e618–e651.
- Farmakis, D., Mueller, C. and Apple, F.S. (2020) High-sensitivity cardiac troponin assays for cardiovascular risk stratification in the general population. *Eur. Heart J.*, **41**, 4050–4056.
- Sheng, J.J. and Jin, J.P. (2016) TNNI1, TNNI2 and TNNI3: Evolution, regulation, and protein structure-function relationships. *Gene*, **576**, 385–394.
- Ingles, J., Goldstein, J., Thaxton, C., Caleshu, C., Corty, E.W., Crowley, S.B., Dougherty, K., Harrison, S.M., McGlaughon, J., Milko, L.V. et al. (2019) Evaluating the clinical validity of hypertrophic cardiomyopathy genes. *Circ. Genomic Precis. Med.*, **12**, 57–64.
- Richard, P., Charron, P., Carrier, L., Ledeuil, C., Cheav, T., Pichereau, C., Benaiche, A., Isnard, R., Dubourg, O., Burban, M. et al. (2003) Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation*, **107**, 2227–2232.
- McCarthy, C.P., Raber, I., Chapman, A.R., Sandoval, Y., Apple, F.S., Mills, N.L. and Januzzi, J.L. (2019) Myocardial injury in the era of high-sensitivity cardiac troponin assays. *JAMA Cardiol.*, **4**, 1034–1042.
- Seferović, P.M., Polovina, M., Bauersachs, J., Arad, M., Gal, T.B., Lund, L.H., Felix, S.B., Arbustini, E., Caforio, A.L.P., Farmakis, D. et al. (2019) Heart failure in cardiomyopathies: a position paper from the Heart Failure Association of the European Society of Cardiology. *Eur. J. Heart Fail.*, **21**, 553–576.
- Lyngbakken, M.N., Røsjø, H., Holmen, O.L., Nygård, S., Dalen, H., Hveem, K. and Omland, T. (2016) Gender, high-sensitivity troponin I, and the risk of cardiovascular events (from the Nord-Trøndelag Health Study). *Am. J. Cardiol.*, **118**, 816–821.
- Lazzeroni, D., Rimoldi, O. and Camici, P.G. (2016) From left ventricular hypertrophy to dysfunction and failure. *Circ. J.*, **80**, 555–564.
- Göser, S., Andrassy, M., Buss, S.J., Leuschner, F., Volz, C.H., Öttl, R., Zittrich, S., Blaudeck, N., Hardt, S.E., Pfitzer, G. et al. (2006) Cardiac troponin I but not cardiac troponin T induces severe autoimmune inflammation in the myocardium. *Circulation*, **114**, 1693–1702.
- Okazaki, T., Tanaka, Y., Nishio, R., Mitsuiye, T., Mizoguchi, A., Wang, J., Ishida, M., Hiai, H., Matsumori, A., Minato, N. et al. (2003) Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med.*, **9**, 1477–1483.
- Hammarsten, O., Mair, J., Möckel, M., Lindahl, B. and Jaffe, A.S. (2018) Possible mechanisms behind cardiac troponin elevations. *Biomarkers*, **23**, 725–734.
- Welsh, P., Preiss, D., Hayward, C., Shah, A.S.V., McAllister, D., Briggs, A., Boachie, C., McConnachie, A., Padmanabhan, S., Welsh, C. et al. (2019) Cardiac troponin T and troponin I in the general population. *Circulation*, **139**, 2754–2764.
- Smith, G.D. and Ebrahim, S. (2003) Mendelian randomization: can genetic epidemiology contribute to understanding environmental determinants of disease? *Int. J. Epidemiol.*, **32**, 1–22.
- Krokstad, S., Langhammer, A., Hveem, K., Holmen, T.L., Midthjell, K., Stene, T.R., Bratberg, G., Heggland, J. and Holmen, J. (2013) Cohort profile: The HUNT study, Norway. *Int. J. Epidemiol.*, **42**, 968–977.
- Taliun, D., Harris, D.N., Kessler, M.D., Carlson, J., Szpiech, Z.A., Torres, R., Taliun, S.A.G., Corvelo, A., Gogarten, S.M., Kang, H.M. et al. (2021) Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. *Nature*, **590**, 290–299.
- Bossard, M., Thériault, S., Aeschbacher, S., Schoen, T., Kunz, S., von Rotz, M., Estis, J., Todd, J., Risch, M., Mueller, C. et al. (2017) Factors independently associated with cardiac troponin I levels in young and healthy adults from the general population. *Clin. Res. Cardiol.*, **106**, 96–104.
- Lyngbakken, M.N., Myhre, P.L., Røsjø, H. and Omland, T. (2019) Novel biomarkers of cardiovascular disease: applications in clinical practice. *Crit. Rev. Clin. Lab. Sci.*, **56**, 33–60.
- Willeit, P., Welsh, P., Evans, J.D.W., Tschiderer, L., Boachie, C., Jukema, J.W., Ford, I., Trompet, S., Stott, D.J., Kearney, P.M. et al. (2017) High-sensitivity cardiac troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants. *J. Am. Coll. Cardiol.*, **70**, 558–568.
- de Lemos, J.A., Drazner, M.H., Omland, T., Ayers, C.R., Khera, A., Rohatgi, A., Hashim, I., Berry, J.D., Das, S.R., Morrow, D.A. et al. (2010) Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. *JAMA*, **304**, 2503–2512.
- Omland, T., De Lemos, J.A., Sabatine, M.S., Christophi, C.A., Rice, M.M., Jablonski, K.A., Tjora, S., Domanski, M.J., Gersh, B.J., Rouleau, J.L. et al. (2009) A sensitive cardiac troponin T assay in stable coronary artery disease. *N. Engl. J. Med.*, **361**, 2538–2547.
- Omland, T., Pfeffer, M.A., Solomon, S.D., De Lemos, J.A., Røsjø, H., Benth, J.Š., Maggioni, A., Domanski, M.J., Rouleau, J.L., Sabatine, M.S. et al. (2013) Prognostic value of cardiac troponin I measured with a highly sensitive assay in patients with stable coronary artery disease. *J. Am. Coll. Cardiol.*, **61**, 1240–1249.
- Chin, C.W.L., Shah, A.S.V., McAllister, D.A., Cowell, S.J., Alam, S., Langrish, J.P., Strachan, F.E., Hunter, A.L., Choy, A.M., Lang, C.C. et al. (2014) High-sensitivity troponin I concentrations are a marker of an advanced hypertrophic response and adverse outcomes in patients with aortic stenosis. *Eur. Heart J.*, **35**, 2312–2321.
- Røsjø, H., Hijazi, Z., Omland, T., Westerbergh, J., Lyngbakken, M.N., Alexander, J.H., Gersh, B.J., Granger, C.B., Hylek, E.M., Lopes, R.D. et al. (2020) Cardiac troponin is associated with cardiac outcomes in men and women with atrial fibrillation, insights from the ARISTOTLE trial. *J. Intern. Med.*, **288**, 248–259.
- Olsson, M., Stanne, T.M., Pedersen, A., Lorentzen, E., Kara, E., Martinez-Palacian, A., Rønnow Sand, N.P., Jacobsen, A.F., Sandset, P.M., Sidelmann, J.J. et al. (2018) Genome-wide analysis of genetic determinants of circulating factor VII-activating protease (FSAP) activity. *J. Thromb. Haemost.*, **16**, 2024–2034.
- Willeit, J., Kiechl, S., Weimer, T., Mair, A., Santer, P., Wiedermann, C.J. and Roemisch, J. (2003) Marburg I polymorphism of factor VII-activating protease: a prominent risk predictor of carotid stenosis. *Circulation*, **107**, 667–670.
- Ahmad-Nejad, P., Dempfle, C.E., Weiss, C., Bugert, P., Borggreve, M. and Neumaier, M. (2012) The G534E polymorphism of the gene encoding the Factor VII-

- activating protease is a risk factor for venous thrombosis and recurrent events. *Thromb. Res.*, **130**, 441–444.
28. Kanai, M., Akiyama, M., Takahashi, A., Matoba, N., Momozawa, Y., Ikeda, M., Iwata, N., Ikegawa, S., Hirata, M., Matsuda, K. et al. (2018) Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat. Genet.*, **50**, 390–400.
  29. Hicks, D., Sarkozy, A., Muelas, N., Köehler, K., Huebner, A., Chinnery, P.F., Barresi, R., Eagle, M. and Polvikoski, T. (2011) A founder mutation in Anoctamin 5 is a major cause of limb girdle muscular dystrophy. *Brain*, **134**, 171–182.
  30. Matsuyama, S., Kage, Y., Fujimoto, N., Ushijima, T., Tsuruda, T., Kitamura, K., Shiose, A., Asada, Y., Sumimoto, H. and Takeya, R. (2018) Interaction between cardiac myosin-binding protein C and formin Fhod3. *Proc. Natl. Acad. Sci. U. S. A.*, **115**, E4386–E4395.
  31. Esslinger, U., Garnier, S., Korniat, A., Proust, C., Kararigas, G., Müller-Nurasyid, M., Empana, J.P., Morley, M.P., Perret, C., Stark, K. et al. (2017) Exome-wide association study reveals novel susceptibility genes to sporadic dilated cardiomyopathy. *PLoS One*, **12**, e0172995.
  32. Pirruccello, J.P., Bick, A., Wang, M., Chaffin, M., Friedman, S., Yao, J., Guo, X., Venkatesh, B.A., Taylor, K.D., Post, W.S. et al. (2020) Analysis of cardiac magnetic resonance imaging in 36,000 individuals yields genetic insights into dilated cardiomyopathy. *Nat. Commun.*, **11**, 2254.
  33. Phelan, D.G., Anderson, D.J., Howden, S.E., Wong, R.C.B., Hickey, P.F., Pope, K., Wilson, G.R., Pébay, A., Davis, A.M., Petrou, S. et al. (2016) ALPK3-deficient cardiomyocytes generated from patient-derived induced pluripotent stem cells and mutant human embryonic stem cells display abnormal calcium handling and establish that ALPK3 deficiency underlies familial cardiomyopathy. *Eur. Heart J.*, **37**, 2586–2590.
  34. Norland, K., Sveinbjornsson, G., Thorolfsdottir, R.B., Davidsen, O.B., Tragante, V., Rajamani, S., Helgadottir, A., Gretarsdottir, S., van Setten, J., Asselbergs, F.W. et al. (2019) Sequence variants with large effects on cardiac electrophysiology and disease. *Nat. Commun.*, **10**, 4803.
  35. Shiraishi, S., Zhou, C., Aoki, T., Sato, N., Chiba, T., Tanaka, K., Yoshida, S., Nabeshima, Y., Nabeshima, Y.I. and Tamura, T.A. (2007) TBP-interacting Protein 120B (TIP120B)/Cullin-associated and Neddylation-dissociated 2 (CAND2) inhibits SCF-dependent ubiquitination of myogenin and accelerates myogenic differentiation. *J. Biol. Chem.*, **282**, 9017–9028.
  36. Jia, L., Shi, Y., Wen, Y., Li, W., Feng, J. and Chen, C. (2018) The roles of TNFAIP2 in cancers and infectious diseases. *J. Cell. Mol. Med.*, **22**, 5188–5195.
  37. Nielsen, J.B., Thorolfsdottir, R.B., Fritsche, L.G., Zhou, W., Skov, M.W., Graham, S.E., Herron, T.J., McCarthy, S., Schmidt, E.M., Sveinbjornsson, G. et al. (2018) Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. *Nat. Genet.*, **50**, 1234–1239.
  38. Inouye, M., Ripatti, S., Kettunen, J., Lyytikäinen, L.P., Oksala, N., Laurila, P.P., Kangas, A.J., Soininen, P., Savolainen, M.J., Viikari, J. et al. (2012) Novel loci for metabolic networks and multi-tissue expression studies reveal genes for atherosclerosis. *PLoS Genet.*, **8**, e1002907.
  39. Björkqvist, J., Jämsä, A. and Renné, T. (2013) Plasma kallikrein: The bradykinin-producing enzyme. *Thromb. Haemost.*, **110**, 399–407.
  40. Nichols, W.M., Seligsohn, U., Zivelin, A., Terry, V.H., Hertel, C.E., Wheatley, M.A., Moussalli, M.J., Hauri, H.P., Ciavarella, N., Kaufman, R.J. et al. (1998) Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII. *Cell*, **93**, 61–70.
  41. Xiong, Y., Wang, L., Mo, P., Huang, G., Li, A., Chai, R., Lin, X., Zhong, Y., Liu, B.B., Ou, W. et al. (2017) Association between HLA-DQB1 alleles and susceptibility to coronary artery disease in Southern Han Chinese. *Hum. Immunol.*, **78**, 540–546.
  42. Köttgen, A., Pattaro, C., Böger, C.A., Fuchsberger, C., Olden, M., Glazer, N.L., Parsa, A., Gao, X., Yang, Q., Smith, A.V. et al. (2010) Multiple new loci associated with kidney function and chronic kidney disease: The CKDGen consortium. *Nat. Genet.*, **42**, 376–384.
  43. Houlihan, L.M., Davies, G., Tenesa, A., Harris, S.E., Luciano, M., Gow, A.J., McGhee, K.A., Liewald, D.C., Porteous, D.J., Starr, J.M. et al. (2010) Common variants of large effect in F12, KNG1, and HRG are associated with activated partial thromboplastin time. *Am. J. Hum. Genet.*, **86**, 626–631.
  44. Verweij, N., Mahmud, H., Leach, I.M., De Boer, R.A., Brouwers, F.P., Yu, H., Asselbergs, F.W., Struck, J., Bakker, S.J.L., Gansevoort, R.T. et al. (2013) Genome-wide association study on plasma levels of midregional-proadrenomedullin and C-terminal-pro-endothelin-1. *Hypertension*, **61**, 602–608.
  45. Williams, T.M. (2001) Human leukocyte antigen gene polymorphism and the histocompatibility laboratory. *J. Mol. Diagnostics*, **3**, 98–104.
  46. Omland, T. (2010) New features of troponin testing in different clinical settings. *J. Intern. Med.*, **268**, 207–217.
  47. Samarel, A.M., Koshman, Y., Swanson, E.R. and Russell, B. (2013) Biophysical forces modulate the costamere and Z-disc for sarcomere remodeling in heart failure. In Solaro, R. and Tardiff, J. (eds), *Biophysics of the Failing Heart. Biological and Medical Physics, Biomedical Engineering*. Springer, New York, pp. 141–174.
  48. Manisastry, S.M., Zaal, K.J.M. and Horowitz, R. (2009) Myofibril assembly visualized by imaging N-RAP, alpha-actinin, and actin in living cardiomyocytes. *Exp. Cell Res.*, **315**, 2126–2139.
  49. Gehmlich, K., Geier, C., Osterziel, K.J., Van Der Ven, P.F.M. and Fürst, D.O. (2004) Decreased interactions of mutant muscle LIM protein (MLP) with N-RAP and  $\alpha$ -actinin and their implication for hypertrophic cardiomyopathy. *Cell Tissue Res.*, **317**, 129–136.
  50. Salazar-Mendiguchiá, J., Ochoa, J.P., Palomino-Doza, J., Domínguez, F., Diéz-López, C., Akhtar, M., Ramiro-León, S., Clemente, M.M., Pérez-Cejas, A., Robledo, M. et al. (2020) Mutations in TRIM63 cause an autosomal-recessive form of hypertrophic cardiomyopathy. *Heart*, **106**, 1342–1348.
  51. van Eldik, W. and Passier, R. (2013) Signalling in sarcomeres in development and disease. *Neth. Hear. J.*, **21**, 367–371.
  52. Shah, S., Henry, A., Roselli, C., Lin, H., Sveinbjornsson, G., Fatemifar, G., Hedman, Å.K., Wilk, J.B., Morley, M.P., Chaffin, M.D. et al. (2020) Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat. Commun.*, **11**, 163.
  53. Fassett, J., Xu, X., Kwak, D., Zhu, G., Fassett, E.K., Zhang, P., Wang, H., Mayer, B., Bache, R.J. and Chen, Y. (2019) Adenosine kinase attenuates cardiomyocyte microtubule stabilization and protects against pressure overload-induced hypertrophy and LV dysfunction. *J. Mol. Cell. Cardiol.*, **130**, 49–58.
  54. Huxley, R., Mendis, S., Zheleznyakov, E., Reddy, S. and Chan, J. (2010) Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk: a review of the literature. *Eur. J. Clin. Nutr.*, **64**, 16–22.
  55. Evans, J.M., Wang, S., Greb, C., Kostas, V., Knapp, C.F., Zhang, Q., Roemmele, E.S., Stenger, M.B. and Randall, D.C. (2017)

- Body size predicts cardiac and vascular resistance effects on men's and women's blood pressure. *Front. Physiol.*, **8**, 561.
56. Panteghini, M. (1990) Aspartate aminotransferase isoenzymes. *Clin. Biochem.*, **23**, 311–319.
  57. Pratt, D.S. and Kaplan, M.M. (2000) Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N. Engl. J. Med.*, **342**, 1266–1271.
  58. Black, P.H. and Garbutt, L.D. (2002) Stress, inflammation and cardiovascular disease. *J. Psychosom. Res.*, **52**, 1–23.
  59. Smith, B.H., Campbell, A., Linksted, P., Fitzpatrick, B., Jackson, C., Kerr, S.M., Deary, I.J., MacIntyre, D.J., Campbell, H., McGilchrist, M. et al. (2013) Cohort profile: Generation Scotland: Scottish family health study (GS: SFHS). The study, its participants and their potential for genetic research on health and illness. *Int. J. Epidemiol.*, **42**, 689–700.
  60. Ferreira, M.A., Vonk, J.M., Baurecht, H., Marenholz, I., Tian, C., Hoffman, J.D., Helmer, Q., Tillander, A., Ullemer, V., Van Dongen, J. et al. (2017) Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat. Genet.*, **49**, 1752–1757.
  61. Nagy, R., Boutin, T.S., Marten, J., Huffman, J.E., Kerr, S.M., Campbell, A., Evenden, L., Gibson, J., Amador, C., Howard, D.M. et al. (2017) Exploration of haplotype research consortium imputation for genome-wide association studies in 20,032 Generation Scotland participants. *Genome Med.*, **9**(1), 23.
  62. Apple, F.S. and Collinson, P.O. (2012) Analytical characteristics of high-sensitivity cardiac troponin assays. *Clin. Chem.*, **58**, 54–61.
  63. Loh, P.R., Tucker, G., Bulik-Sullivan, B.K., Vilhjálmsson, B.J., Finucane, H.K., Salem, R.M., Chasman, D.I., Ridker, P.M., Neale, B.M., Berger, B. et al. (2015) Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat. Genet.*, **47**, 284–290.
  64. Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, **26**, 2190–2191.
  65. Yang, J., Lee, S.H., Goddard, M.E. and Visscher, P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.*, **88**, 76–82.
  66. Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W. et al. (2010) Common SNPs explain a large proportion of heritability for human height. *Nat. Genet.*, **42**, 565–569.
  67. Wang, K., Li, M. and Hakonarson, H. (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.*, **38**, e164.
  68. Wang, G., Sarkar, A.K., Carbonetto, P. and Stephens, M. (2020) A simple new approach to variable selection in regression, with application to genetic fine mapping. *J.R. Statist. Soc B*, **82**, 1273–1300.
  69. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R., Willer, C.J. and Friseman, D. (2011) LocusZoom: Regional visualization of genome-wide association scan results. *Bioinformatics*, **27**, 2336–2337.
  70. Pers, T.H., Karjalainen, J.M., Chan, Y., Westra, H.J., Wood, A.R., Yang, J., Lui, J.C., Vedantam, S., Gustafsson, S., Esko, T. et al. (2015) Biological interpretation of genome-wide association studies using predicted gene functions. *Nat. Commun.*, **6**, 5890.
  71. Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M. and Haussler, A.D. (2002) The Human Genome Browser at UCSC. *Genome Res.*, **12**, 996–1006.
  72. Giambartolomei, C., Vukcevic, D., Schadt, E.E., Franke, L., Hingorani, A.D., Wallace, C. and Plagnol, V. (2014) Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet.*, **10**, e1004383.
  73. R Core Team (2019) R: A Language and Environment for Statistical Computing. *R foundation for Statistical Computing*, Vienna, Austria. <https://www.R-project.org/>
  74. Manichaikul, A., Mychaleckyj, J.C., Rich, S.S., Daly, K., Sale, M. and Chen, W.M. (2010) Robust relationship inference in genome-wide association studies. *Bioinformatics*, **26**, 2867–2873.
  75. Wei, W.Q., Bastarache, L.A., Carroll, R.J., Marlo, J.E., Osterman, T.J., Gamazon, E.R., Cox, N.J., Roden, D.M. and Denny, J.C. (2017) Evaluating phecodes, clinical classification software, and ICD-9-CM codes for phenotype-wide association studies in the electronic health record. *PLoS One*, **12**, e0175508
  76. Zhou, W., Nielsen, J.B., Fritsche, L.G., Dey, R., Gabrielsen, M.E., Wolford, B.N., LeFaive, J., VandeHaar, P., Gagliano, S.A., Gifford, A. et al. (2018) Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nat. Genet.*, **50**, 1335–1341.
  77. Hemani, G., Zheng, J., Elsworth, B., Wade, K.H., Baird, D., Haberland, V., Laurin, C., Burgess, S., Bowden, J., Langdon, R. et al. (2018) The MR-Base platform supports systematic causal inference across the human phenome. *eLife*, **7**, e34408.
  78. Hemani, G. (2020) MRInstruments: data sources for genetic instruments to be used in MR. 2020. R package version 0.3.2. <https://github.com/MRCIEU/MRInstruments/>
  79. Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M. et al. (2015) UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.*, **12**, e1001779.
  80. Aung, N., Vargas, J.D., Yang, C., Cabrera, C.P., Warren, H.R., Fung, K., Tzanis, E., Barnes, M.R., Rotter, J.I., Taylor, K.D. et al. (2019) Genome-wide analysis of left ventricular image-derived phenotypes identifies fourteen loci associated with cardiac morphogenesis and heart failure development. *Circulation*, **140**, 1318–1330.