Association analyses based on false discovery rate implicate many new loci for coronary artery disease

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Genome-wide association studies (GWAS) in coronary artery disease (CAD) have identified 66 loci at 'genome-wide significance' ($p < 5 \times 10^{-8}$) but a much larger number of putative loci at a false discovery rate (FDR) of 5%[1-4]. Here, we leverage an interim release of UK Biobank (UKBB) data to evaluate the validity of the FDR approach. We tested a CAD phenotype inclusive of angina (SOFT; N_{cases}=10,801) as well as a stricter definition without it (HARD; N_{cases}=6,482) and selected the former for conducting a meta-analysis with the two most recent CAD GWASs[2-3]. This approach identified 13 new loci at genome-wide significance, 12 of which were in our previous 5% FDR list[2], and provided strong support that the remaining FDR loci represent genuine signals. The set of 304 independent variants at 5% FDR in this study explain 21.2% of CAD heritability and identified 243 loci that implicate pathways in blood vessel morphogenesis as well as lipid metabolism, nitric oxide signaling and inflammation.

Previous GWAS studies of CAD risk[1-4] have interrogated a large number of cases and controls but remain less well-powered than GWAS of quantitative traits[5]. UKBB was established to improve understanding of the causes of common diseases including CAD, a leading health problem around the world[6]. In addition to self-reported disease outcomes and extensive health and life-style questionnaire data, the 502,713 participants are being tracked through their NHS records and national registries (including cause of death and Hospital Episode Statistics). In July 2015, UKBB released genotypes imputed to the 1000 Genomes panel for 152,249 participants profiled with a SNP array harboring 820,967 variants comprising common variants optimized for imputation, validated rare coding variants and sets of phenotype-associated variants or their proxies (e.g. GWAS catalogue).

We set up The UKBiobank-CardioMetabolic-Consortium CHD working group to assess the use of self-reported and hospital record data on CAD in UKBB and define the relevant case and control subgroups to undertake genetic analyses of CAD risk.

The July 2015 release of UKBB comprises 10,801 genotyped individuals with an inclusive CAD phenotype ('SOFT') that incorporates self-reported angina or other evidence of chronic coronary heart disease, of which 6,482 have a more stringently defined CAD phenotype ('HARD') of myocardial infarction and/or revascularisation (**Fig. 1a**). After QC we analysed the SOFT and HARD cases separately against 137,914 controls for 9,149,595 variants present either in the CARDIoGRAMplusC4D 1000-Genomes GWAS[2] or the MIGen/CARDIoGRAM Exome-chip study[3-4]. The SOFT definition was selected for the primary analysis based on power calculations (**Supplementary Table 1**). We found 4 (SOFT and HARD), 1 (SOFT only) and 2 (HARD only) variants reaching genome-wide significance, all located in known CAD loci (**Supplementary Figure 1**).

We then meta-analysed the UKBB data for each CAD definition with each of the two published data sets (**Supplementary Figure 2**) using an inverse-variance weighted fixed-effect (IVW-FE) model and double genomic control correction (**Online Methods**). For both the SOFT and HARD definitions, we validated all 66 known CAD loci (72 independent variants with $p < 1.2x10^{-3}$ IVW-FE) with 43 and 37 respectively reaching genome-wide significance in this study (**Supplementary Table 2**). Outside the known CAD loci (1 Mb window centred on the published lead SNP) we found 9 new signals (in both SOFT and HARD) reaching genome-wide significance (**Table 1** and **Fig. 2**). The anticipated increase in power with the SOFT definition (**Supplementary Table 1**) was attenuated by an inflation of the lambda statistic (**Supplementary Table 3**), potentially due to a combination of larger sample size (i.e. polygenicity) and a less homogeneous phenotype in the SOFT definition. Overall, there was

strong concordance between corresponding signals for SOFT and HARD (**Fig. 1b, Supplementary Table 4**); subsequent analyses were undertaken using the SOFT meta-analysis results.

To look for additional signals beyond the 9 that reached genome-wide significance (**Fig. 2**) we performed an FDR analysis and selected 23 suggestive signals at 1% FDR ($p < 1.55x10^{-6}$ IVW-FE; **Supplementary Table 4**) outside known CAD loci which we validated in an independent sample of up to 4,412 cases and 3,910 controls from the German MI-Family-Studies V and VI and a Greek case-control study (**Supplementary Table 5**). In total, we identified 13 new genome-wide significant CAD loci in the combined discovery and replication sample (**Table 1, Supplementary Table 6**).

In our recent large-scale GWAS[2], we reported 162, mainly common, variants at an FDR discovery cutoff of 5% showing conditional independent associations with the P_{joint} test in GCTA[7]. Twelve of the 13 new sentinel SNPs were present or had a proxy ($r^2>0.8$) among these 162 variants[2]. **Fig. 3** shows a strong linear relationship between association signals for these 162 variants in the earlier[2] and current analysis, with overall greater significance levels in the current meta-analysis. As expected, we observed an excess of small p-values for this set of variants in the UK Biobank alone (**Supplementary Figure 3a**). Monte Carlo simulations show that the expected number of replicated variants in the UK Biobank data is 56 (95% CI 42 – 69) (**Supplementary Figure 3b**) and we found 58 variants after allowing for multiple testing (q-values < 0.05). This further confirms the validity of extended lists of associated variants based on FDR criteria. We therefore defined a new FDR list of association signals by performing an approximate joint association analysis with the GCTA software[7] as described elsewhere[2] using the 11,427 SNPs with 5%FDR. We identified 304 independent variants at $P_{joint} < 10^{-4}$, clustering in 243 putative CAD loci (**Supplementary Table 7**). The new 5%FDR set

overlaps by 122 SNPs with the old set (75.3%; including proxies at an $r^2 > 0.8$). We then assessed heritability using the independent set of 304 SNPs and obtained a heritability estimate of 21.2%. The contribution to this heritability estimate of the 13 new loci (**Table 1**) was 1.03% whereas the new and known genome-wide significant CAD loci together explained 8.53% of CAD heritability. To further assess the validity and utility of the 5%FDR set, we tested the ability to predict CAD using genetic risk scores (GRS) based on either the 5%FDR SNPs (GRS1) or only CAD variants reaching genome-wide significance (GRS2; **Online Methods**) in an independent sample, EPIC-CVD[8], comprising 7910 CHD cases and 12958 controls. In a model with age and sex, GRS1 increased the C-index by 0.25% compared to GRS2 (**Supplementary Table 8**). GRS1 improved the point estimates of the HR compared to GRS2 mainly in the second (from 0.9116 to 0.8314) and fourth quintile (from 1.0437 to 1.176), **Supplementary Figure 4**.

We then explored the biology of the 13 new genome-wide significant CAD risk loci; **Supplementary Figure 5** shows regional association plots**. Supplementary Figure 6** provides *in silico* functional annotation (**Online Methods**) for each lead variant and its proxies (1000 Genomes). We found compelling evidence to implicate candidate genes *ITGB5*, *TGB1*, *PDE5A*, *ARHGEF26*, *FN1*, *CDH13*, and *HNF1* (detailed in **Supplementary Note)**. The risk allele of rs150512726 (proxy for rs142695226; **Table 1**), causes a 3 amino acid deletion within the cytoplasmic tail of integrin subunit beta 5 (ITGB5), part of a heterodimer which regulates the activation of latent TGFB1 (Transforming growth factor beta 1)[9-10]. The intronic variant (rs8108632; **Table 1**) we identified in *TGFB1*, further implicates the TGFB1 pathway in CAD risk. TGFB1 is known to have important roles in endothelium and vascular smooth muscle[11] but has not been widely studied in atherosclerosis, though a recent study implicates TGF-β signalling downstream of CDKN2B in the *CDKN2BAS* cardiovascular risk locus[12]. eQTL

analyses suggested candidate CAD risk genes (*TDRKH*, *FN1*, *ARHGEF26*, *PDE5A*, *ARNTL*, and *CDH13*) in six new loci (**Supplementary Table 9**). For example, the lead variant rs7678555 (**Table 1**) was found to be a strong eQTL ($p=8.1x10^{-13}$ linear regression model) for *PDE5A* only in aorta from CAD patients (STARNET[13]; **Supplementary Table 9**) although its regulatory potential was modest using functional prediction tools (**Online methods**). *PDE5A* encodes a cGMP-specific phosphodiesterase which is important for smooth muscle relaxation in the cardiovascular system where it regulates nitric-oxide-generated cGMP[14]. Furthermore, mining eQTL data in tissues from CAD patients (STARNET) showed several other instances of eSNPs (*TDRKH*, FN1, *CDH13*; **Supplementary Table 9**) having no effect in tissues from non-CAD patients (GTEx[15]). One caveat is that sample size differs between STARNET and GTex for certain tissues. Nonetheless, our observation highlights the need to expand efforts to map regulatory elements in disease tissues.

Other candidate genes fit with emerging data on atherosclerosis mechanisms. For example, a knockout mouse for *ARHGEF26* on a hyperlipidemic background resulted in reduced atherosclerosis and plaques with reduced macrophage content[16]. Similarly, *FN1* expression is increased in plaques and mouse models have demonstrated a causal role for fibronectin-1 in the development and progression of atherosclerosis[17-18]. Finally, we undertook a phenome scan to assess pleiotropy (**Supplementary Table 10**). Several of the new lead SNPs (or a proxy) had robust associations ($p < 5x10^{-8}$ meta-analysis) with traditional CAD risk factors such as LDL-cholesterol (*HNF1A* and *FN1*), blood pressure (*PRDM8/FGF5*) and BMI (*SNRPD2*).

We next evaluated the broader functional relationships among genes associated with variants (N=11,427) at 5%FDR. The 5%FDR set was annotated for eQTLs which, when

present, were mainly found in atherosclerotic aortic wall (25%) or internal mammary artery (22%) of CAD patients (STARNET[13]; **Supplementary Table 9**). In GTEx[15], eQTLs were mainly found in subcutaneous fat (**Supplementary Table 9; Supplementary Figure 7**).

Prior pathway analyses of GWAS CAD loci have highlighted genes involved in lipid metabolism, cellular movement, and processes such as tissue morphology and immune cell trafficking[1]. Analysis of 357 genes, selected as either eQTLs and/or the nearest gene to a 5%FDR independent variant in this study (N=304), with the Ingenuity Knowledge base confirmed the above findings[1] highlighting cardiovascular system development and function ($p = 1.31x10^{-16}$ right-tailed Fisher Exact Test (rtFET)), organismal development ($p = 1.31 \times 10^{-16}$ rtFET) and survival ($p = 1.52x10^{-16}$ rtFET) as the most significant processes. In addition to canonical pathways related to lipid metabolism, extracellular matrix, inflammation and nitric oxide production, the 357 gene set showed enrichment for angiogenesis and signalling by the pro-angiogenic growth factor VEGF (**Supplementary Figure 8**). We also applied DEPICT[19] with the full distribution of 5%FDR signals (**Online Methods**) to search for enriched gene sets. Blood vessel development, which includes angiogenesis, was in the top 10 ($p < 6.67x10^{-12}$ enrichment test[19]) DEPICT Grouped-GeneSets (GO:0001568; **Fig. 4, Supplementary Figure 9, Supplementary Table 11**).

Ingenuity built 5 networks out of the 357 genes with the largest three integrating 12 of the new candidate CAD risk genes with 67 candidate genes in known CAD loci (**Supplementary Table 12**). In total, the 5 networks comprise 66.4% of the 357 genes.

This is the largest CAD genetic study to assess simultaneously common and rare (MAF < 1%)/low-frequency (MAF 1-5%) variants. In total, 101 low-frequency and 3 rare variants reached genome-wide significance among all 5%FDR markers (N=11,427). This apparent paucity in rare variants which has also been reported for type 2 diabetes[20], is likely due to

lack of power compared to studies of quantitative traits e.g. a study of adult height in ~700,000 individuals has reported 32 rare variants[5]. As expected, lower-frequency variants tend to have stronger effects compared to common variants (**Supplementary Figure 10**) with the exception of rs2891168 in *CDK2NB-AS1* (MAF 48.7%; OR 1.19; **Supplementary Table 13**). The intergenic variant rs186696265 which had the largest OR (1.62) in our study is known to affect LDL cholesterol levels[21].

Our findings highlight the importance of the FDR approach to define an extended list of associated variants. As we have previously proposed[1-2], suggestive association signals in well-powered GWAS such as this one can substantially improve our knowledge of disease architecture at only a modest penalty implied by the 5%FDR. We have demonstrated the potential value of the new 5%FDR list in improving prediction of CAD risk and implicating new networks underlying CAD pathophysiology. This extended list of candidate genes provides a powerful resource for functional studies.

We note that while this work was in review a study was published also reporting associations of the *HNF1A* locus with CAD[22].

URLs

www.ukbiobank.ac.uk/

GWAS catalogue: https://www.ebi.ac.uk/gwas/

GTEx portal: http://www.gtexportal.org/home/

PhenoScanner: http://www.phenoscanner.medschl.cam.ac.uk/

Ingenuity Knowledge Base: http://www.ingenuity.com/science/knowledge-base?utm_source=Blog&utm_medium=link&utm_campaign=Doug%20Bassett%20ASHG%202014

281 https://biobank.ctsu.ox.ac.uk/crystal/docs/genotyping_qc.pdf
282 http://cnsgenomics.com/shiny/INDI-V/

283 http://www.broadinstitute.org/mpg/depict

284 https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/

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AUTHOR CONTRIBUTIONS

Writing group (wrote and edited manuscript): C.P.N., A.G., A.S.B., S.K., T.R.W., E.M., I.N., J.C.H., O.G., H.S., M.F., J.D., N.J.S., H.W., P.D. All authors contributed and discussed the results, and commented on the manuscript. Data generation & cohorts: A.S.B., O.G., T.J., L.Z., S.E.H., E.A., T.L.A., E.P.B., J.C.C., R.C., R.M.C., P.E., R.E., E.E., P.W.F., C.G., D.G., A.H., J.M.M.H., E.I., A.K., T.Ke., T.Ky., T.L., X.L., Y.L., W.M., R.McP., A.M., C.N.A.P., M.Pujades-R., A.F.S., M.J.S., P.A.Z., K. A., R.J.F.L., E.Z., J.E., G.D., H.S., J.D., N.J.S., H.W., P.D. Phenotype data (UK Biobank, replication): C.P.N., A.S.B., I.N., F.Y.L., J.C.H., O.G., B.D.K., J.S.K., R.J.F.L., R.S.P., M.R., M.T., I.T., E.Z., J.E., G.D., H.S., J.D., N.J.S., H.W., P.D. Statistical analysis: C.P.N., A.G., A.S.B., S.K., T.J., M.F. Functional annotation: C.P.N., S.K., T.R.W., A.S.B., R.E., A.R., E.E.S., J.L.M.B. Biological and clinical enrichment and pathway analyses: E.M., P.D.

COMPETING FINANCIAL INTERESTS

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Figure legends

Figure 1. Description of HARD and SOFT CAD phenotypes in UK Biobank. (**a**) Diagram depicting the CAD phenotype definition in UK Biobank. HARD CAD defined as fatal or non-fatal myocardial infarction (MI), PTCA (percutaneous transluminal coronary angioplasty), or coronary artery bypass grafting (CABG). SOFT CAD includes HARD CAD as well as chronic ischaemic heart disease (IHD) and angina. UK Biobank self-reported data: 'Vascular/heart problems diagnosed by doctor' or 'Non-cancer illnesses that self-reported as angina or heart attack'. Self-reported surgery defined as either PTCA, CABG or triple heart bypass. HESIN hospital episodes data and death registry data using diagnosis and operation - primary and secondary cause: MI defined as hospital admission or cause of death due to ICD9 410-412, ICD10 I21-I24, I25.2; PTCA is defined as hospital admission for PTCA (OPCS-4 K49, K50.1, K75); CABG is defined as hospital admission for CABG (OPCS-4 K40 – K46); Angina or chronic IHD defined as hospital admission or death due to ICD9 413, 414.0, 414.8, 414.9, ICD10 I20, I25.1, I25.5-I25.9. (**b**) Radar plot highlighting the proportions (%) of signals between the HARD and SOFT CAD phenotype definitions based on the 5%FDR results (**Supplementary Table 4)**; MAF = minor allele frequency, $p < 5x10^{-8}$ marks variants reaching genome-wide significance, OR = odds ratio (OR > 1.05 corresponds to 85% power to detect a signal (alpha < 0.05) in the SOFT analysis). The results for all six subgroups of variants assessed did not differ statistically between the two phenotype definitions (p>0.1)

Figure 2. Transposed Manhattan plot showing the SOFT meta-analysis results under an additive model. The *P*-values are truncated at –log10(*P*) = 20. Markers shown are from the meta-analysis of UK Biobank with the 1000G GWAS data[2] unless flagged by an * (exome chip markers). The red dotted lines are at GWAS (P=5x10^{-8}) and 5% FDR significance (P=6.28x10^{-5}). The known CAD risk loci are shown in black (**Supplementary Table 2**); *KSR2* and *ZNF507*-

LOC400684 had reached genome-wide significance under a recessive model[2]. The 11p15_MRVI1 / CTR9 locus had discordant results between the CAD 1000 Genomes GWAS[2] and Exome[4] data set. The lead variant in the Exome data set, rs11042937, had $P = 3.21 \times 10^{-8}$; data shown are from the meta-analysis with the 1000Genomes GWAS as this marker had an imputation info score of 1 (Online Methods). The 13 novel CAD loci which reached genome-wide significance in our study (including replication data; **Table 1**), are written in brown font.

Figure 3. Single marker p-value comparison of the 5% FDR variants in the published CARDIoGRAMplusC4D 1000Genomes CAD GWAS meta-analysis[2] and current FDR study. Of the 162 variants which had $p < 5 \times 10^{-5}$ in the CAD 1000Genomes GWAS, 116 had a match or good proxy ($r^2 > 0.8$) in the new FDR list (blue circles). SNPs in red (n=7) were present in the earlier FDR list and reached genome-wide significance in the current analysis.

Figure 4. Heat map showing the DEPICT gene set enrichment results with zoom-in on a subset of the results. 556 gene sets are included which had evidence of enrichment at 1% FDR. The x– axis shows the gene name, which is predicted to be included in the reconstituted gene set indicated in the y – axis. The color red indicates higher Z-score, where Z-score is a value representing each gene's inclusion in DEPICT's reconstituted gene sets. Clustering was made based on complete linkage method. Highlighted pathways in the cluster, include angiogenesis, blood vessel development and morphogenesis.

Table 1-Novel variants reaching genome-wide significance ($P<5x10^{-8}$) in the combined (discovery and replication) SOFT meta-analysis

Locus Name	Markername	CHR	POS (hg38)	EA	EAF	Functional Evidence	UKBB+CoG/Exome				Meta analysis		
							OR	95% CI	Pvalue	FDR Qvalue	OR	95% CI	Pvalue
TDRKH	rs11810571	1	151762308	G	0.849	eQTL/coding	1.060	1.039-1.082	$2.21x10^{-8}$	$8.05x10^{-5}$	1.057	1.036-1.079	$4.24x10^{-8}$
FN1	rs1250229*	2	216304384	T	0.256	eQTL/coding	1.072	1.052-1.092	$1.85x10^{-13}$	$2.05x10^{-9}$	1.071	1.051-1.091	$2.77x10^{-13}$
RHOA	rs7623687	3	49448566	A	0.855	none	1.074	1.049-1.100	$3.72x10^{-9}$	$1.62x10^{-5}$	1.076	1.052-1.101	$3.44x10^{-10}$
UMPS/ITGB5	rs142695226	3	124475201	G	0.138	eQTL/coding	1.069	1.045-1.094	$1.00x10^{-8}$	$3.98x10^{-5}$	1.071	1.048-1.095	$1.53x10^{-9}$
ARHGEF26	rs12493885*	3	153839866	C	0.886	eQTL	1.074	1.047-1.101	$3.29x10^{-8}$	$1.15x10^{-4}$	1.073	1.047-1.101	$3.16x10^{-8}$
PRDM8/FGF5	rs10857147	4	81181072	T	0.275	none	1.056	1.036-1.075	$8.96x10^{-9}$	$3.60x10^{-5}$	1.054	1.036-1.073	$5.66x10^{-9}$
PDE5A/MAD2L1	rs7678555	4	120909501	C	0.301	eQTL	1.049	1.031-1.069	$1.43x10^{-7}$	$4.25x10^{-4}$	1.052	1.034-1.070	$1.32x10^{-8}$
HDGFL1	rs6909752	6	22612629	A	0.351	none	1.051	1.034-1.069	$5.59x10^{-9}$	$2.35x10^{-5}$	1.051	1.034-1.068	$2.19x10^{-9}$
ARNTL	rs3993105	11	13303071	T	0.704	none	1.048	1.030-1.067	$1.06x10^{-7}$	$3.33x10^{-4}$	1.048	1.031-1.066	$4.77x10^{-8}$
HNF1A	rs2244608	12	121416988	G	0.355	coding	1.053	1.035-1.070	$2.32x10^{-9}$	$1.06x10^{-5}$	1.053	1.035-1.070	$7.74x10^{-10}$
CDH13	rs7500448	16	83045790	A	0.752	eQTL	1.061	1.040-1.082	$5.14x10^{-9}$	$2.18x10^{-5}$	1.063	1.043-1.083	$4.76x10^{-10}$
TGFB1	rs8108632	19	41854534	T	0.488	none	1.049	1.031-1.067	$5.88x10^{-8}$	$1.95x10^{-4}$	1.048	1.031-1.066	$4.04x10^{-8}$
SNRPD2	rs1964272	19	46190268	G	0.510	none	1.045	1.028-1.063	$2.29x10^{-7}$	$6.15x10^{-4}$	1.047	1.030-1.064	$2.46x10^{-8}$

*Exome marker

EA: effect allele; EAF: Effect allele frequency; CoG = CARDIoGRAMplusC4D 1000G GWAS; Exome = Exome array analysis; UKBB = UK Biobank; Discovery sample comprised 71,602 cases and 260,875 controls (for exome markers 53,135 and 215,611 respectively); Replication sample comprised up to 4412 cases and 3910 controls. Functional evidence for the locus is given where the lead variant or a variant in high LD ($r^2>0.8$) is a coding change, has evidence as an expression quantitative trait locus (eQTL), or both. Further details of functional evidence are provided in **Supplementary Table 7** and **Supplementary Figure 6**.

Online Methods

Phenotype Definitions & Power calculation

UKBB recruited 502,713 individuals aged 40-69 years from England, Scotland and Wales between 2006 and 2010 (94% of self-reported European ancestry). HARD CAD was defined as fatal or non-fatal myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass grafting (CABG). SOFT CAD includes all HARD CAD as well as chronic ischemic heart disease (IHD) and angina. Controls were defined as patients which were not a SOFT case after exclusions (listed below). All conditions were defined by either self-reported, hospital episode or death registry data.

Exclusions were made for aneurysm and atherosclerotic cardiovascular disease using hospital admissions, or cause of death, codes ICD9 414.1, ICD 10 I25.0, I25.3, I25.4, and not having MI, PTCA, CABG, Angina or chronic IHD as defined above.

Susceptibility effect sizes in MI cases and an inclusive CAD definition were very similar in the earlier GWAS[2]. We hypothesized that the detailed clinical information in UKBB might enhance the search for novel loci by further broadening the CAD phenotype to increase sample size.

GWAS and meta-analyses

All participants gave written consent for participation in genetic studies, and the protocol of each study was approved by the corresponding local research ethics committee or institutional review board. Participating cohorts in the 1000 Genomes and Exome GWAS studies are described elsewhere[2,3]. UK Biobank (UKBB samples) were excluded due to withdrawn consent, sex mismatches (n=182), Biobank/Believe QC exclusions (n=406) and sample relatedness (n=3,481) determined as Kinship>0.088. GWAS analysis in UKBB was restricted to variants with results available in the published GWAS[2] or Exome[3-4] dataset. Further exclusions included poorly imputed (info<0.4) or monomorphic variants, duplicate variants across data sets, variants that deviated strongly from Hardy-Weinberg Equilibrium in European ancestry controls ($p<1x10^{-9}$), variants with an effect allele frequency in European ancestry samples that differed strongly (i) from 1000G European panel, (ii) from GWAS/Exome data, (iii) between arrays (UKBB vs UK-BiLEVE), and (iv) across genotyping batches. Variants that did not produce a valid result or estimated extreme log odds ratios (|beta|>4) were also excluded after analysis. Cluster plots lead variants and of proxies were visually inspected.

We ran the GWAS under an additive frequentist mode of inheritance for each variant using the dosages from the imputed data, adjusting for array (UK Biobank vs UK BiLEVE) and the first five principal components (see **URLs**) using SNPTEST. Age and sex were not adjusted for to maximize the power to detect associations with diseases that have a prevalence <10%[23]. Population stratification was assessed and standard errors were adjusted using the genomic inflation statistic (λ).

Association summary statistics (after λ correction) from the UKBB were combined with the 1000 Genomes (1000G) imputed GWAS results[2] and the Exome results[3] via two separate fixed-effect inverse-variance weighted meta-analysis implemented in GWAMA[24]. We applied post meta-analysis λ correction in each instance. We identified 36,460 variants present in both the 1000G imputed GWAS and the Exome results. We

retained the variants from the 1000G imputed GWAS if the median info score was 1, otherwise we retained the results from the Exome data.

Comparison of SOFT vs HARD peak variant lists at 5% q-value

The false discovery rate (FDR) following the meta-analysis with UKBB was assessed using a step-up procedure in the *qqvalue* Stata program[25] as it is well controlled under positive regression-dependency conditions. We used the Simes method to generate q-values for the 8.9M variants. The p-value cut-off for a q-value of 5% for HARD was 7.24×10^{-5} and SOFT was 6.28×10^{-5}. Peak SNPs were identified in a 1cM window. There is an exact overlap of 155 variants between the 2 peak variant lists, however, using the 1cM window the overlap increases to 206 variants. Both the lists were annotated and classified into 6 categories (exome chip, indels, Odds Ratio (OR)>1.05, p<5e-8, MAF<5% and exonic). The proportions were calculated in each of the 6 categories and plotted as a radar plot (**Fig. 1b**). Monte Carlo simulations were used to assess the *post-hoc* power of the UKBB interim data to replicate the 155 variants. The 1000G GWAS effect sizes ("betas") are expected to be subject to *winner's curse* inflation so were shrunken (towards the null) by application of the FIQT procedure[26]. Effect sizes for firmly established CAD loci were systematically lower for SOFT compared to the HARD phenotype (**Supplementary Table 1**) noting that HARD closely corresponds to the CAD phenotype in reference 2. Betas were therefore further shrunken by a factor log (1.059)/log(1.072) = 0.82 (**Supplementary Table 1**). 10,000 replicates were then randomly drawn from the vector of shrunken betas and the corresponding UKBB standard errors, to allow for variation in genotype call rates, imputation quality and allele frequency and to calculate Wald association statistics. Multiple testing of 155 variants was allowed for by controlling the FDR to 5% with a step-up procedure encoded in the *multproc*[27] Stata™ program. The average expected number of replicated variants was 56 (95%CI 42 – 69). Testing the 5% FDR variants (**Supplementary Table 7**) in UKBB with a model adjusted for age and sex gave concordant results to the unadjusted model (data not shown).

GCTA & Heritability analysis

We used the GCTA software[7] to perform joint association analysis in (SOFT) meta-analysis results. This approach fits an approximate multiple regression model using summary-level meta-analysis statistics and LD corrections estimated from a reference panel (here the UKBB sample). We adopted a chromosome-wide stepwise selection procedure to select variants and estimate their joint effects at i) a genome-wide significance level (pJoint ≤ 5×10^{-8}) in the totality of meta-analysed variants (n~ 9M; **Supplementary Figure 10**, **Supplementary Table 11**) and ii) a Bonferroni-corrected pJoint<1×10^{-4} corresponding to the number of independent LD bins ($r^2 < 0.1$) in the 5% FDR variant list (n=11,427; **Supplementary Table 6**).

Heritability calculations were based on a multifactorial liability-threshold model, implemented in the INDI-V[28] calculator (see **URLs**), under the assumption of a baseline population risk (K) of 0.0719[29] and a twins heritability (H_L^2) of 0.4. Multiple regression estimates from the GCTA joint association analysis were used to estimate heritability for the 304 independent CAD risk variants within the 5% FDR list.

Genetic risk score analysis

GRS analysis was undertaken in the EPIC-CVD study[8] which comprises 7910 CAD cases and 12958 controls (**Supplementary Note**). We considered either all known and new lead CAD risk variants reaching genome-wide significance (GRS2; **Supplementary Table 2** and **Table 1**) or the 304 variants in the 5% FDR set (GRS1; **Supplementary Table 7**). We used variants with an INFO score filter of 0.4 in EPIC-CVD and replaced missing ones with proxies (r2 > 0.8 in 1000 Genomes European participants). GRS1 comprised 280 variants and GRS2 71. The raw GRS was obtained by summing the dosages of these variants for all individuals. We then fitted a Prentice weighted cox regression model for each GRS, adjusting for age and sex, to obtain survival forecasts and calculate the C indices. Statistical analyses were performed using R 3.3.3 and STATA 13.1. Variant extraction was done using qctool 1.4.

Functional annotation

eQTLs: For associations between the 304 independent variants (5% FDR) and gene expression traits we searched for expression quantitative trait loci (eQTLs) in the Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task (STARNET) RNA-seq dataset[13] and the Genotype-Tissue Expression[15] (GTEx) portal. eQTLs were included if the best eSNP (i.e. the variant with the most significant association with gene expression in cis) was in high LD (r^2>0.8) with the CAD lead SNP.

Regulatory elements: We functionally annotated each of the 13 lead variants and their proxies (r^2>0.8) using HaploregV4[30]. Overlap with regulatory elements including chromosome state segmentation, DNase hypersensitivity, and transcription factor binding (TFB) as determined by the ENCODE[31] and Roadmap Epigenome projects[32], and predicted effects on TFB based on regulatory motifs from TRANSFAC[33] and JASPAR[34] were identified using HaploregV4[19] and the UCSC genome browser. Variants were then scored using three different bioinformatics tools that help prioritise causal disease variants. Combined Annotation Dependent Depletion (CADD)[35] incorporates a range pathogenicity prediction tools to provide a genome-wide score (C-score) for each test variant from its pre-calculated database of ~8.6 billion genetic variants. High scores indicate variants that are not stabilized by selection and are more likely to be disease-causing and low scores indicate evolutionary stable non-damaging variants. The top 10% of likely functional variants will have a C-score >10 and top 1% of variants will have a C-score >20. Genome-wide annotation of variants (GWAVA)[36] predicts the functional impact of noncoding variants based on genomic and epigenomic annotations and provides scores between 0 and 1 with higher scores indicating variants that are more likely to be functional. RegulomeDB[37] annotates and scores variants in seven categories based datasets such as ENCODE. Scores of 1-2 variants likely to affect TFB, 3 less likely to affect binding, 4-6 relate to variants with minimal binding evidence and 7 is for variants with no regulatory annotation.

Phenome-scan: look ups in other common traits were performed using the PhenoScanner database as described in reference 38.

Pathway analysis

DEPICT: DEPICT[19] is a computational tool which performs gene set enrichment analyses to prioritize genes in associated GWAS loci with probabilistically predefined gene sets based on Gene Ontology terms, canonical pathways, protein-protein interaction subnetworks and rodent phenotypes; reconstituted gene sets are detailed in references

19 and 39. Input to our analysis were the 11,427 CAD variants (FDR 5%) of which 11,311 were annotated in DEPICT. We constructed loci as previously described (beta version 1.1, release 194, see **URLs**). Analysis was performed with default parameters (50 repetitions to compute FDRs, 500 permutations to adjust for biases, such as gene length). The 11,311 variants were collapsed to 288 loci which were used in the gene set enrichment analyses. Correlated gene sets were grouped together based on gene membership to expedite data interpretation.

Ingenuity: Genes were selected using 304 independent SNPs (5% FDR) based on eQTLs (**Supplementary Table 9**) and physical proximity (included overlapping genes on opposite strands or at equal distance from the SNP). Spliced ESTs and putative transcripts were not included. Network analysis was performed using the Ingenuity Pathway Analysis software (see **URLs**). We considered molecules and or relationships available in The IPA Knowledge Base (IKB) for human OR mouse OR rat and set the confidence filter to Experimentally Observed OR High (Predicted). Networks were generated with a maximum size of 70 genes and up to 10 networks were allowed. Networks are ranked according to their degree of relevance to the 'eligible' molecules in the query data set. The network score is based on the hypergeometric distribution and is calculated with the right-tailed Fisher's Exact Test. The significance p-value associated with enrichment of functional processes is calculated using the right-tailed Fisher Exact Test by considering the number of query molecules that participate in that function and the total number of molecules that are known to be associated with that function in the IKB.

Data Availability Statement: Meta-analysis summary statistics for all variants considered in this study for association with CAD (SOFT definition) are available at http://www.cardiogramplusc4d.org/data-downloads/.

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