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Analysis of rare genetic variation underlying cardiometabolic diseases and traits among 200,000 individuals in the UK Biobank

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Abstract

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Author Contributions Statement

S.J.J., S.H.C., S.A.L. and P.T.E. conceived and designed the study. S.J.J., S.H.C., V.N.M., M.C., J.P.P and J.L.H. performed data curation and data processing, for data other than the MyCode dataset. S.J.J., S.H.C. and V.N.M. performed statistical analyses, for data other than the MyCode dataset. M.T.O., B.L., D.P.v.M. and C.M.H. performed data curation, data processing and statistical analyses in the MyCode dataset. S.J.J., S.H.C. and M.C. performed data visualization. K.G.A., K.L.L., S.A.L. and P.T.E. supervised the overall study. S.J.J., S.H.C. and P.T.E. drafted the manuscript. L.-C.W., V.N., C.R. and A.W.H. contributed critically to the analysis plan and revisions of the manuscript. All authors critically revised and approved the manuscript. Contributions from consortium members from the Regeneron Genetics Center are provided in the Supplementary Note.

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#These authors jointly supervised this work.

Competing Interests Statement

P.T.E. has received sponsored research support from Bayer AG and IBM Research. P.T.E. has also served on advisory boards or consulted for Bayer AG, MyoKardia and Novartis. S.A.L. receives sponsored research support from Bristol Myers Squibb / Pfizer, Bayer AG, Boehringer Ingelheim, Fitbit, and IBM, and has consulted for Bristol Myers Squibb / Pfizer, Bayer AG, and Blackstone Life Sciences. L.-C.W. is supported by a grant from IBM to the Broad Institute. The remaining authors have no relevant competing interests to disclose.

Cardiometabolic diseases are the leading cause of death worldwide. Despite a known genetic component, our understanding of these diseases remains incomplete. Here we analyzed the contribution of rare variants to 57 diseases and 26 cardiometabolic traits, using data from 200,337 UK Biobank participants with whole-exome sequencing. We identified 57 gene-based associations, with broad replication of novel signals in Geisinger MyCode. There was a striking risk associated with mutations in known Mendelian disease genes, including *MYBPC3*, *LDLR*, *GCK*, *PKD1* and *TTN*. Many genes showed independent convergence of rare and common variant evidence, including an association between *GIGYF1* and type 2 diabetes. We identified several large-effect associations for height, and 18 unique genes associated with blood lipid or glucose levels. Finally, we found that between 1.0 and 2.4% of participants carried rare potentially pathogenic variants for cardiometabolic disorders. These findings may facilitate studies aimed at therapeutics and screening of these common disorders.

Over the past decade, genome-wide association studies (GWAS) have provided critical insights into the genetic architecture of cardiometabolic traits and diseases, through the identification of thousands of associated genomic loci[1-7]. Typically, these studies focused on common variants, which individually often confer small effect sizes and do not always directly implicate causal genes. Familial linkage and targeted sequencing studies have identified numerous Mendelian causes of cardiovascular disease[8-11], although such studies have been limited by small sample size. A handful of larger case-control studies have had some success in discovery of genes harboring large-effect variants for adult-onset disease, for example, for myocardial infarction[12] and diabetes[13].

Analysis of large-scale population-based sequencing data offers multiple advantages over conventional common variant association studies. First, sequencing provides the opportunity to identify rare and ultra-rare genetic variants, which often would not have been genotyped, or typed inaccurately, by array genotyping and imputation[14]. Second, exome sequencing—which is focused on the protein-coding regions of the genome—may directly implicate genes in phenotype variability through burden testing of multiple rare protein-coding variants[15]. Third, analysis of rare coding variation can help establish the directionality of impaired gene function through the analysis of loss-of-function alleles, a feature that can be informative both for understanding disease mechanisms and for potential therapeutic targeting. In cardiovascular research, a paradigmatic example of this approach is *PCSK9*, which progressed from gene discovery to an available therapeutic in just over a decade[16-18]. Finally, sequencing at scale enables assessment of penetrance, risk, and carrier frequencies for rare deleterious genetic variation[14,19].

Here we present an analysis of the second release of whole-exome sequencing data from the UK Biobank, a population-based study, consisting of sequencing data on approximately 200,000 individuals[20]. Through exome-wide gene-based analyses, we evaluate the contributions of rare (minor allele frequency (MAF) < 0.1%) damaging variants to 83 traits, including anthropometric traits, cardiometabolic diseases, metabolic blood biomarkers, electrocardiographic traits and cardiac magnetic resonance imaging traits. We replicate novel signals in the Geisinger MyCode cohort (also known as the DiscovEHR study), comprising 166,000 participants with exome sequencing data. Finally, we describe

the frequency of mutations in genes underlying cardiovascular diseases and monogenic diabetes.

Results

Exome-wide rare variant analysis of 83 traits identifies 57 significant associations.

After sample level quality control procedures, we identified 200,337 UK Biobank participants with a mean age of 57 years at enrollment and 68 years at last follow-up, of which 55% were female and 87% were of white-British European ancestry (Supplementary Fig. 1). Table 1 provides the baseline characteristics and case number for each representative disease phenotype. A total of 12,756,075 distinct autosomal genetic variants were available from the exome sequencing data after quality control, of which 12,553,131 had a MAF < 0.1%.

We performed exome-wide gene-based tests across 57 medical conditions (Supplementary Tables 1 and 2) and 26 quantitative cardiometabolic traits (Supplementary Table 3), testing rare loss-of-function (LOF) and missense variants. Exome-wide gene-based analyses showed good calibration of *P*-values across all performed tests (Supplementary Figs. 2-4 and Supplementary Note), although three traits (height, weight and QTc) showed moderate inflation of test statistics ($1.1 \leq \lambda < 1.25$; Supplementary Fig. 4). In an attempt to identify the cause of the inflation, we analyzed rare synonymous variants. Exome-wide analysis of rare synonymous variation produced good calibration of test statistics (Supplementary Fig. 5), indicating that a large proportion of the observed inflation was due to polygenicity rather than bias. We then performed a number of additional sensitivity analyses. When we restricted our analyses to individuals of homogeneous white-British European ancestry, all identified gene-phenotype associations still showed strong evidence of association with comparable effect size estimates (Supplementary Tables 4 and 5 Supplementary Figs. 6 and 7). Furthermore, when restricting to LOF variants only, we generally observed comparable effect size estimates across the significant associations (Supplementary Figs. 8 and 9). One notable exception was the association between *GCK* and type 2 diabetes, for which effect sizes were attenuated by the inclusion of predicted-deleterious missense variants.

In total, we identified 57 significant associations across 83 traits at an overall FDR Q < 0.01, which was equivalent to $P < 5.44 \times 10^{-7}$. All tests reaching Q < 0.05 are displayed in Supplementary Tables 4 and 5. Of the 57 significant associations, 42 were known, while 15 represented novel associations (Tables 2 and 3). Given the many parallel analyses of the same dataset, we define ‘novel’ to indicate rare variant associations that were not described prior to the release of the UK Biobank exomes. Across the disease associations where rare variants were associated with increased disease risk, the median OR was large at 5.6 (Q1-Q3: 2.4-13.0). The median absolute beta for significant quantitative associations was 0.63 s.d. (Q1-Q3: 0.43-0.85).

Identified associations represent burdens of multiple rare variants and are independent of common variation.

To assess the stability of our results to changes in the analytic approach, we performed a leave-one-variant-out (LOVO) analyses for each of the significant associations. For example, when analyzing the relation between *TSHR* and hypothyroidism, the highest LOVO *P*-value attained for the significant binary associations was $P = 7.9 \times 10^{-4}$ after removing p.Trp546Ter (Supplementary Tables 4 and 6). Similarly, the highest LOVO *P*-value for the significant quantitative associations was $P = 5.7 \times 10^{-4}$ for *LCAT*/high-density lipoprotein after removing p.Tyr107Ter (Supplementary Tables 5 and 7). Importantly, the novel associations that we identified remained robust in a LOVO analysis (Supplementary Fig. 10). Thus, the genes significantly associated with diseases or traits were identified due to a burden of multiple contributing rare variants, although in certain cases—such as the associations of *ANGPTL2* with height and *NR1H3* with high-density-lipoprotein—single variants were important. The complete LOVO results for all variants, as well as variant frequencies and annotations, are presented in Supplementary Tables 6 and 7.

We then aimed to evaluate whether the identified rare variant signals were independent of nearby common variants. We first performed common variant analyses for common (MAF > 0.5%) imputed variants within ± 500 kb of the gene; we then performed the rare variant association tests with the index common variants as additional covariates (Methods). Overall, we found that the effect size estimates, and *P*-values, were not significantly changed after conditioning on nearby common variants (Supplementary Tables 8 and 9). Interestingly, the association of *LPA* with lipoprotein(a) became more significant ($P = 4.3 \times 10^{-10}$, $\beta = -0.35$; $P_{conditional} = 7.7 \times 10^{-23}$, $\beta_{conditional} = -0.45$), as did the suggestive associations *KCNQ1* with QTc, *BSN* with BMI, and *BSN* with weight ($P_{conditional} < 5.44 \times 10^{-7}$; Supplementary Table 9).

Independent replication in the Geisinger MyCode cohort.

To replicate gene-based associations that we identified in the UK Biobank, we utilized data from the Geisinger MyCode cohort from the Geisinger health system[21] (also known as the DiscovEHR cohort). MyCode is a healthcare-based cohort with high quality whole-exome sequencing data for 166,661 adults (aged 57 ± 18 years, 61% female) of primarily European ancestry (95%) linked to longitudinal electronic health records. We put forward the 15 associations that were unreported prior to the release of UK Biobank exomes. Of 14 testable associations, 13 (93%) replicated in MyCode at $P < 0.05$ with consistent direction of effect (Table 4).

Rare variants confer substantial risk and penetrance for cardiometabolic and other disorders.

From the exome-wide analyses of 57 curated medical conditions in the UK Biobank, we observed a number of well-described gene-disease associations (Fig. 1 and Table 2). For cardiac diseases, these include the association between dilated and hypertrophic cardiomyopathy with the genes *TTN* and *MYBPC3,* respectively. For metabolic disorders involving cholesterol transport, glucose regulation, and thyroid disorders, associations were noted with *LDLR*, *APOB*, *PCSK9*, *GCK*, *TSHR* and *TG*. Interestingly, biallelic mutations in

TSHR and *TG* are known to cause penetrant congenital thyroid disease (MIM 275200 and 274700), while our findings indicate that heterozygote carriers may also be at 2 to 3-fold increased odds of hypothyroidism. Accordingly, large-effect missense variants in both genes have been found to affect thyroid stimulating hormone levels[22], and common variants near both genes are associated with thyroid disease, including autoimmune thyroiditis[23].

In many cases, the phenotypic penetrance of deleterious variants and the increased risk of disease was striking. For example, 71% of *LDLR* mutation carriers had hypercholesterolemia (OR 13.1, 95%CI 8.3-21.3), 45% of *MIP* variant carriers had cataracts (OR 7.6, 95%CI 3.4-16.7) and 47% of *PKD1* variant carriers had chronic kidney disease (OR 40.3, 95%CI 21.3-76.2) (Supplementary Table 10 and Supplementary Figs. 11 and 12). Indeed, *PKD1* mutations, while known for penetrant autosomal dominant polycystic kidney disease, have recently been suggested to exhibit incomplete penetrance[24] (Supplementary Note). *GCK* mutation carriers had over 14-fold increased odds (95%CI 8.3-23.4) of type 2 diabetes, with over 48% of carriers having a diagnosis. *GCK* variants are known to cause maturity-onset diabetes of the young[25] and have also previously been found enriched in type 2 diabetes cases[26,27] (Supplementary Note). *TTN* mutations were associated with a several-fold increased risk of multiple cardiovascular disorders, including an over 11-fold increased odds of dilated cardiomyopathy (MIM 604145), and a more than doubling in the risk of heart failure, atrial fibrillation[19], and ventricular arrhythmia[28]. *TTN* variants also had novel associations with the risk of supraventricular tachycardia (OR 2.5, $P = 2.9 \times 10^{-9}$) and mitral valve disease (OR 2.3, $P = 2.6 \times 10^{-11}$), findings which were replicated in MyCode (OR 1.4, $P = 0.01$ and OR 1.5, $P = 8.3 \times 10^{-4}$, respectively). While these specific associations have not been reported before, they may be related to diagnoses of atrial fibrillation or dilated cardiomyopathy. Of note, all known and novel *TTN* associations became stronger (markedly higher ORs and lower *P*-values) after restricting to exons highly expressed in cardiac left ventricular tissue (Supplementary Note).

Rare variants in *GIGYF1* are associated with diabetes risk.

We further identified two gene associations for diabetes, which were not reported prior to the release of UK Biobank exomes. Rare mutations in *GIGYF1* were significantly associated with an increased risk of type 2 diabetes (55 carriers, OR 5.6, $P = 3.0 \times 10^{-7}$), and also associated significantly with higher blood glucose levels ($\beta = 0.8$ s.d., $P = 9.5 \times 10^{-9}$) and lower low-density lipoprotein levels ($\beta = -0.8$ s.d., $P = 9.02 \times 10^{-9}$) (Tables 2 and 3 and Fig. 3). *GIGYF1* further showed suggestive evidence of association with lower insulin-like growth factor-1 levels (Fig. 3). Common variants near *GIGYF1* also associated with diabetes risk and glucose levels[7] (Supplementary Table 11). Using GTEx, we found that the top common variants in this locus were strong expression-QTLs for *GIGYF1* in multiple relevant tissues (adipose tissue, pancreas, thyroid, skeletal muscle), with the expression-lowering alleles showing consistency with the observed LOF association (Supplementary Note and Supplementary Table 12). The protein product of *GIGYF1* regulates insulin-like-growth factor signaling[29] and interacts with Grb10, a protein that has been implicated in insulin signaling, glucose tolerance and insulin resistance[30]. *GIGYF1* LOFs have previously been linked to autism[31], although none of the carriers in the present study had ICD code diagnoses relevant to autism or developmental delay. In MyCode, the association between

rare variants in *GIGYF1* and type 2 diabetes was replicated (OR 3.2, $P = 2.0 \times 10^{-9}$), as was the association with glucose ($\beta = 0.5$, $P = 1.4 \times 10^{-9}$). We further leveraged summary data from a previous exome sequencing study of diabetes[13], and found additional evidence of independent replication for *GIGYF1* LOF variants (9 carriers, OR 8.6, $P = 7.8 \times 10^{-3}$). Across all three studies, *GIGYF1* variants were robustly associated with type 2 diabetes (OR 3.8, $P = 4.1 \times 10^{-16}$; Extended Data Fig. 1).

Mutations in *CCAR2*, also known as *KIAA1967* and *DBC1*, were also associated with diabetes risk in the UK Biobank (26 carriers, OR 12.8, $P = 5.4 \times 10^{-8}$). Common variants near *CCAR2* associated with diabetes risk as well[7] (Supplementary Table 11), and the top common variant in this locus was found to be a significant expression-QTL for *CCAR2* in multiple relevant tissues (Supplementary Table 12 and Supplementary Note). Previous studies have suggested that *CCAR2* regulates a glucose metabolic gene network, and that the gene is downregulated in cells from diabetic patients[32]. Furthermore, *Ccar2* knockout mice develop a metabolic phenotype, including obesity, elevated glucose and insulin resistance[33]. However, we were not able to replicate the association between *CCAR2* rare variants and diabetes in MyCode ($P = 0.62$). Therefore, the role for *CCAR2* remains uncertain, and further studies are necessary to dissect the contribution of *CCAR2* mutations to human diabetes.

Rare variants confer large effect sizes for quantitative cardiometabolic and anthropometric traits.

In our primary analyses, we identified 18 unique genes that were significantly associated with blood lipid or glucose levels (Table 3 and Fig. 2). Rare variants conferred large effect sizes, ranging from 0.3 to 2.2 s.d., and in many cases showed pleiotropy across multiple metabolic traits (Fig. 3 and Supplementary Table 13). As expected, *APOB* ($\beta = -2.2$ s.d. for low-density lipoprotein and $\beta = -1.4$ s.d. for triglycerides), *APOC3* ($\beta = -1.2$ s.d. for triglycerides) and *GCK* ($\beta = 1.2$ s.d. for glucose) were among genes conferring the largest effect sizes.

Our analysis revealed several genes that have been proposed as potential therapeutic targets or for which lipid-lowering therapeutics are in development, such as *ANGPTL3*[34], *ANGPTL4*[35] and *PCSK9*[16-18]. Other notable findings included *PDE3B*, in which damaging variants were associated with lower triglyceride levels ($\beta = -0.3$ s.d., $P = 3.6 \times 10^{-7}$), consistent with previous reports[4]. *PDE3B* variants have also been associated with improved body fat distribution, making it an interesting therapeutic target[36]. Of note, our findings are independent of previous UK Biobank analyses for this gene, as the functional[4,36] stop-gain variant p.Arg783Ter was not included in the current analysis based on MAF filters. Indeed, conditioning on p.Arg783Ter, and nearby common variants, did not strongly affect the results ($\beta = -0.3$ s.d., $P = 4.3 \times 10^{-6}$) (Supplementary Table 9).

We further found that rare mutations in *PLIN1* were associated with elevated high-density lipoprotein levels ($\beta = 0.4$ s.d., $P = 8.01 \times 10^{-15}$), an association that was confirmed in MyCode ($\beta = 0.4$ s.d., $P = 9.29 \times 10^{-5}$). Furthermore, *PLIN1* variants associated suggestively with decreased triglyceride levels ($\beta = -0.2$ s.d., $P = 1.6 \times 10^{-5}$) and nominally with lower risk of hypercholesterolemia (OR 0.69, $P = 9.0 \times 10^{-3}$) and lower risk of

coronary artery disease (OR 0.56, $P = 0.03$) (Fig. 3 and Supplementary Table 13). *PLIN1* frameshift variants have paradoxically been linked to a phenotype of partial lipodystrophy with low levels of high-density lipoprotein and high triglycerides[37]. Our findings, however, are consistent with the notion that *PLIN1* haploinsufficiency does not cause partial lipodystrophy[38] and indicate that *PLIN1* inhibition[39] might represent a potential target for lipid therapeutics.

Among the many associations between rare variants and cardiometabolic traits, lipid associations for *GIGYF1* (low-density lipoprotein; $\beta = -0.8$ s.d., $P = 9.02 \times 10^{-9}$) and *NR1H3* (high-density lipoprotein; $\beta = 0.4$ s.d., $P = 3.27 \times 10^{-16}$) were also novel, and replicated in MyCode (*GIGYF1*: $\beta = -0.3$ s.d., $P = 1.76 \times 10^{-3}$; *NR1H3*: $\beta = 0.4$ s.d., $P = 2.97 \times 10^{-6}$, respectively). Common variants near both genes show concordant associations with blood lipid levels[40,41] (Supplementary Table 11). Furthermore, top common variants in both loci are significant expression- or splice-QTLs for these genes in relevant tissues (Supplementary Table 12 and Supplementary Note). *NR1H3* encodes the liver X receptor alpha, a regulator of cholesterol homeostasis in the liver[42]. For *GIGYF1*, our findings indicate that damaging variants may be beneficial for blood lipids (e.g. lower low-density lipoprotein), yet harmful for glucose homeostasis (e.g. higher glucose and increased risk of diabetes). Interestingly, an inverse association between cholesterol and diabetes risk has been observed previously for common variants[43,44], and for lipid medications such as statins[45,46]. A multivariate GWAS analyzing diabetes and concurrent lower low-density lipoprotein in the UK Biobank previously identified a locus overlapping *GIGYF1*[47].

We further found 7 novel rare variant associations for height (Table 3 and Fig. 2), all of which replicated at $P < 0.05$ in MyCode (Table 4). While common and low-frequency variant analyses have already implicated *DTL*, *ZFAT*, *PIEZO1*, *SCUBE3*, *ANGPTL2*, *IRS1* and *PAPPA* in standing height[48-50] (Supplementary Table 11 and Supplementary Note), our results indicate that rare variation in these genes may confer substantial effects, with absolute effect sizes ranging from 0.2 to 1.0 s.d. (Tables 3 and 4). Interestingly, *IRS1* and *ANGPTL2* represent genes of possible interest to cardiometabolic health. *Irs1* knockout mice exhibit both impaired growth and insulin resistance[51], while *ANGPTL2* is involved in many metabolic processes, including cardiac energy metabolism and heart failure[52].

Over 1% of individuals carry putatively pathogenic rare variants for cardiometabolic disease.

Given the high prevalence and morbidity of cardiometabolic disease in the general population, we then sought to quantify carrier frequencies and disease associations for putatively pathogenic variation in the UK Biobank. Among 71 cardiovascular disease associated genes included on typical sequencing panels for arrhythmias, cardiomyopathies and hypercholesterolemia, we identified 55 genes that were reported for autosomal dominant Mendelian inheritance (Supplementary Table 14). Similarly, we found 13 genes reported for dominant forms of adult-onset diabetes. For each gene, we collapsed carrier status for rare LOF, pathogenic and likely pathogenic variants (Methods) and performed association tests with relevant diseases. Genes associated with a relevant disease at $Q < 0.01$ ($P < 3.0 \times 10^{-4}$) included *TTN*, *MYBPC3*, *MYH7*, *LDLR*, *DSP*, *SCN5A*, *PKP2*, *GCK* and *HNF1A* (Fig. 4

and Extended Data Fig. 2). The median OR for significant associations was 4.5 (Q1-Q3: 3.9-18.8) and became OR 8.2 (Q1-Q3: 4.1-22.9) after excluding *TTN* associations.

As expected, variants in *TTN* were most common, as 0.42% of the samples (840 carriers) carried a truncating variant located in one of the cardiac exons (Fig. 4 and Supplementary Table 15). *MYBPC3* variants, which associated with hypertrophic cardiomyopathy (OR 88.9, $P = 2.2 \times 10^{-26}$) and several related phenotypes (Supplementary Table 16), were carried by 0.12% (244 carriers). Putatively pathogenic variants in *LDLR* were observed in 0.12% of individuals (236 carriers) and showed associations with coronary artery disease (OR 3.7, $P = 6.8 \times 10^{-8}$), and myocardial infarction (OR 4.0, $P = 1.5 \times 10^{-6}$). *PKP2* variants, carried by 0.12% of individuals (235 carriers), showed an association with ventricular arrhythmias (OR 4.4, $P = 2.21 \times 10^{-4}$). *SCN5A* (0.10%) and *DSP* (0.06%) showed associations with conduction defects and dilated cardiomyopathy, respectively. *GCK* (0.02%) and *HNF1A* (0.01%) both were associated with type 2 diabetes (Extended Data Fig. 2).

The penetrance of putatively pathogenic cardiovascular disease variants was generally modest (<15%), especially when compared to previous estimates from family-member based analyses, although *GCK* and *HNF1A* variants conferred high absolute risks of diabetes (64 and 44% penetrance, respectively) (Supplementary Note, Supplementary Tables 10 and 15, and Supplementary Fig. 13). The yield of relevant LOFs and known pathogenic variants among disease cases was low for common diseases such as diabetes and atrial fibrillation (generally <5%), while dilated and hypertrophic cardiomyopathy both had rare variant yields of greater than 10% (Supplementary Note, Supplementary Fig. 14 and Supplementary Table 17).

Overall, 2.4% of samples ($n = 4{,}855$) carried a putatively pathogenic variant in any of the 68 panel genes included in our analysis (Fig. 4, Extended Data Fig. 2 and Supplementary Table 15). This statistic includes a number of genes with limited or disputed evidence of pathogenicity (e.g *KCNE2*), as well as LOFs for a number of genes where truncation is not an established mechanism of dominant disease (e.g. *MYL2*, *MYL3*, *MYL4*). As such, this number represents an upper-bound estimate. We then restricted our analysis to genes which associated significantly with a relevant phenotype at $Q < 0.01$. When restricting only to associated genes, we arrive at a lower-bound estimate of 2,098 carriers, or 1.0% of samples, as carriers of putatively pathogenic variants for cardiometabolic disease.

Discussion

The availability of exome sequencing data in nearly 200,000 individuals from the UK Biobank has provided an unparalleled opportunity to explore the genetic basis of common diseases using many distinct analytic approaches[53-55]. Through exome-wide gene-based analysis of very rare genetic variants, we replicate many known Mendelian gene-trait associations for cardiometabolic disorders in the UK Biobank. We also identify several large-effect associations that were not previously reported prior to the release of the UK Biobank exome data, and which were broadly replicated in the independent Geisinger MyCode cohort. We further quantify the frequency of rare pathogenic variation and

show that between 1.0% and 2.4% of individuals carry potentially high-impact putatively pathogenic rare variants for cardiovascular diseases and diabetes.

Our findings permit a number of conclusions. First, our findings show the value of large-scale population sequencing for identifying key contributors to cardiometabolic disease, as well as the relative odds of disease associated with Mendelian mutations. For example, through exome-wide analyses, we identified large effect associations for rare *MYBPC3* variants with hypertrophic cardiomyopathy (OR 120, MIM 115197), *LDLR* mutations with hypercholesterolemia (OR 13, MIM 143890), *PKD1* mutations with chronic kidney disease (OR 40, MIM 173900) and *GCK* with diabetes (OR 14, MIM 125853). In a targeted analysis of panel genes, we further found markedly increased disease risk (OR > 5) for multiple genes, including *TTN*, *MYH7, DSP*, *SCN5A* and *HNF1A.* Our data also allowed estimation of rare variant penetrance, strengths and limitations of which are discussed in detail in the Supplementary Note. These results highlight the potential of large-scale population-based sequencing for assessment of risk and pathogenicity associated with genes and variants.

Second, we identified associations for large-effect coding variants with cardiometabolic traits, which were not reported prior to the release of large-scale exome data. Rare variants in *GIGYF1* were associated with marked increased risk of type 2 diabetes in discovery and replication datasets, with ORs ranging from 3.2 to 8.6. While *GIGYF1* is among the hundreds of loci identified for diabetes through GWAS[7], previous human genetic studies have not explicitly prioritized this gene prior to release of the UK Biobank exomes. In contrast, our findings directly implicate *GIGYF1*, a known regulator of insulin-like growth factor signalling[29], in the pathogenesis of human diabetes. We also highlight novel large-effect associations for standing height, which showed similar convergence between evidence from rare and common genetic variants. We further identified several rare variant associations for blood lipids that have not been previously described through population-based association testing, including *GIGYF1*, *NR1H3* and *PLIN1*, as well as genes which have been put forward as potential therapeutic targets or for which therapeutics are under development, such as *PDE3B*, *ANGPTL3* and *ANGPTL4.* Taken together, these results show the added value of exome sequencing for identifying genes with important roles in disease pathogenesis and therapeutic targeting.

Third, we quantify carrier frequencies of rare pathogenic variants for cardiometabolic diseases and show that a meaningful proportion of individuals carry genetic variants underlying cardiovascular disease and diabetes. LOF, pathogenic or likely pathogenic variants in cardiomyopathy, arrhythmia, hypercholesterolemia or diabetes genes were carried by 2.4% of individuals. Even when restricting to genes that show evidence of association with relevant outcomes, we identify 1.0% of UK Biobank participants as carriers of disease-causing variation. Consistent with previous reports, *TTN* LOF variants were relatively common, accounting for nearly half of this group or 0.42% of individuals[19,56,57]. However, another ~0.5-2% of individuals may carry deleterious variation in other cardiometabolic disease genes. A previous analysis in a smaller subset of the UK Biobank already showed that ~2% of individuals carry clinically-actionable variants in 59 important Mendelian disease genes[14]. Our results were focused on a larger list of cardiometabolic disease genes, while incorporating population-based associations. These studies show the potential for

large-scale sequencing to identify a meaningful proportion of individuals at high risk of disease morbidity and mortality.

Fourth, our results have several analytical implications for rare variant analysis in large biobanks. We found that our rare variant associations were not negatively affected by adjustment for nearby common variation, likely due to our strict variant filter at MAF < 0.1% in both the UK Biobank and gnomAD[58], as employed previously[15]. In addition, effect sizes for LOF and missense variants were comparable to the effect sizes for LOF variants alone, which is likely a reflection of both variant frequency filters and strict inclusion filters based on 30 *in silico* missense prediction tools. Finally, we note that our gene-based implementation of the saddle point approximation controlled well for test statistic inflation, even for extremely imbalanced phenotypes; we employed Firth's regression to yield accurate OR estimates for rare variants. Indeed, recent analytical developments have shown the value of these methods for genetic analyses in large biobanks[59,60]. We have made our code for gene-based burden testing using the saddle point approximation, based on GENESIS code[61], available through the repository https://github.com/seanjosephjurgens/UKBB_200KWES_CVD.

In spite of the large sample size of over 200,000 sequenced individuals, we note that rare variant discovery power was still limited. For example, we identified only 3 associations for type 2 diabetes and 1 for atrial fibrillation at test-wide significance, despite having over 12,000 cases for both phenotypes. This may reflect a modest contribution of rare variants to phenotypic variability, or of a distributed contribution over many genes. In support of the latter, a previous exome sequencing study of type 2 diabetes[13] estimated that over 75,000 sequenced cases, or over 600,000 samples from population-based biobanks, would be necessary to identify known diabetes drug targets at 80% statistical power. Therefore, UK Biobank analyses utilizing data from all 500,000 samples may prove particularly fruitful for complex diseases in the future.

Our study has several other potential limitations. First, participants in the UK Biobank are largely middle-aged individuals of European ancestry. As such, our findings may not be broadly applicable to all age strata and ancestries. Second, disease status in the UK Biobank relies on self-reports, ICD codes, operation codes, and death registry codes. As a consequence, some misclassification for disease phenotypes is possible. However, previous efforts using the same phenotypic definitions in GWAS for a number of analyzed diseases replicated well-described genetic loci for common variants[2,62,63]. Furthermore, many of the exome-wide significant rare variant associations presented here are well-described Mendelian gene-trait associations. Third, there is potential for ascertainment bias among participants in the UK Biobank, making it unlikely that the study perfectly reflects the overall middle-aged UK population. The ascertainment of UK Biobank participants would be anticipated to attenuate rather than inflate effect sizes and penetrance estimates, as discussed in detail in the Supplementary Note. Fourth, we acknowledge that alternate methods for defining diseases or traits are feasible such as analyzing all ICD or Phecodes. However, we used a set of curated disease definitions that builds from prior work and has in many cases been validated and replicated[5,19,64].

In conclusion, large-scale sequencing has enabled the dissection of the rare genetic contributors to cardiometabolic traits and diseases. We confirm many Mendelian gene-disease associations in an unselected, population-based cohort. Furthermore, we also identified and replicated novel large-effect associations for several traits, including diabetes, blood lipids and standing height. Finally, we found that a considerable portion of individuals carry putatively pathogenic variants in cardiomyopathy, arrhythmia, hypercholesterolemia and diabetes genes. In the future, our findings may facilitate studies aimed at therapeutics and screening of cardiovascular and metabolic disorders.

Methods

Study population and phenotypes.

The UK Biobank is a large population-based prospective cohort study from the United Kingdom with deep phenotypic and genetic data on approximately 500,000 individuals aged 40-69 at enrollment[103]. Curated disease phenotypes were defined using reports from medical history interviews, in- and outpatient ICD-9 and -10 codes, operation codes, and death registry records (Supplementary Table 1). For diseases and medical conditions, case status was determined at last follow-up. The breakdown of prevalent and incident cases, as well as the mean age at disease onset, are presented in Supplementary Table 2. Age at disease onset was defined as the earliest of either (i) age when electronic health records or death registry records first reported billing codes, or (ii) age at second or third visit if defined during a UK Biobank visit. Phenotypes defined at the first (baseline) UK Biobank visit were considered missing for age at onset. The UK Biobank further provides access to a wide range of other phenotypic data, including anthropometric measurements, electrocardiographic intervals, metabolic biomarkers, and cardiac magnetic resonance imaging data. The UK Biobank resource was approved by the UK Biobank Research Ethics Committee and all participants provided written informed consent to participate. Use of UK Biobank data was performed under application number 17488 and was approved by the local Massachusetts General Hospital Institutional Review Board.

Sequencing and quality control.

Whole-exome sequencing was performed on over 200,000 participants from the UK Biobank[20], for which the methods have been described for the earlier release of data from approximately 50,000 individuals[14]. The revised version of the IDT xGen Exome Research Panel v1.0 was used to capture exomes with over 20X coverage at 95% of sites. Because the 200K dataset released by the UK Biobank had been subject to limited quality-control and filtering, we applied an extensive genotype, variant and sample level pipeline to produce a high-quality dataset for analysis, for which the methods are described in detail in the Supplementary Note. Briefly, we set low-quality genotypes to missing, after which we removed variants based on call rate (<90%), Hardy-Weinberg equilibrium test ($P < 1 \times 10^{-15}$), presence in low-complexity regions, and minor allele count (≥1). Sample-level quality-control consisted of removal of samples that had withdrawn their consent, were duplicates, had a mismatch between exome sequencing and genotyping array data, had a mismatch between genetically inferred and self-reported sex, had low call rates or were outliers (outside 8 standard deviations from the mean) for a number of additional metrics

(Supplementary Note). Of the 200,642 individuals with exome sequencing who passed the internal quality-control, we excluded an additional 305 samples based on our filters, leaving 200,337 individuals. We also defined an unrelated subset of this cohort (Supplementary Note), which included 185,990 individuals.

Variant annotation.

The protein consequences of variants were annotated using dbNSFP[104] (version 4.1a) and the Loss-of-Function Transcript Effect Estimator[58] (LOFTEE) plug-in implemented in the Variant Effect Predictor[105] (VEP; version 95) (https://github.com/konradjk/loftee). VEP was used to ascertain the most severe consequence of a given variant for each gene transcript. LOFTEE was implemented to identify high-confidence loss-of-function variants (LOF), which include frameshift indels, stopgain variants and splice site disrupting variants. We also removed any LOFs flagged by LOFTEE as dubious, such as LOFs affecting poorly conserved exons and splice variants affecting NAGNAG sites or non-canonical splice regions. Missense variants were annotated using 30 *in silico* prediction tools included in the dbNSFP database. We collapsed information from the 30 tools into a single value, representing the percentage of tools which predicted a given missense variant was deleterious (Supplementary Note). A missense variant was considered damaging if at least 90% of *in silico* prediction tools predicted it to be deleterious.

Rare variant burden analyses.

To identify genes and rare genetic variation relevant to cardiometabolic diseases and traits, we performed association tests between a curated binary or quantitative phenotype and rare variants using gene-based collapsing tests. Variants were considered rare if they had minor allele frequency (MAF) <0.1% in the UK Biobank exome sequencing dataset, and <0.1% in each major continental population in gnomAD[58] (version 2 exomes). In our primary analysis, we collapsed carriers of LOF variants and predicted-damaging missense variants into a single variable by sample, for each gene. For a given binary phenotype, genes with ≥20 rare variant carriers were analyzed using a logistic mixed-effects model implemented in GENESIS[61] (version 2.18.0), adjusting for age, sex, sequencing batch (first 50K vs remaining 150K) and significantly associated ($P < 0.05$) ancestral principal components (Supplementary Note). For analyses of MRI data, we additionally adjusted for MRI serial number. Missing genetic data were imputed to zero. We accounted for relatedness by including a sparse kinship matrix as a random effect (Supplementary Note), and *P*-values were computed using the saddle point approximation to account for case-control imbalance[106]. Odds ratios (OR) and confidence intervals for binary traits were estimated using Firth's bias-reduced logistic regression[107] in the unrelated subset of the cohort. Quantitative traits were inverse-rank normalized and analyzed using a linear mixed-effects model in GENESIS, implementing the same fixed and random effects and using score tests. For two traits, high-density lipoprotein and lipoprotein(a), the mixed-effects nullmodel failed to converge. We therefore ran these traits using standard linear regression in the unrelated subset of the cohort.

We conducted exome-wide association tests for a curated set of 57 binary disease phenotypes and 26 quantitative traits. The binary disease phenotypes have an emphasis

on cardiac diseases, vascular disease, metabolic disorders, and also include a range of additional conditions (Supplementary Table 2). We further analyzed 26 quantitative traits, including anthropometric measurements, metabolic blood markers, electrocardiographic intervals and magnetic resonance imaging traits. Anthropometric (including height, weight, body mass index, systolic blood pressure and diastolic blood pressure), metabolic biomarker data (high-density lipoprotein, low-density lipoprotein, triglycerides, glucose, insulin-like-growth-factor 1) and pulse rate measurements were available in a range of 150,000 and 200,000 individuals. Approximately 22,700 individuals had 12-lead resting electrocardiographic data available, including the RR interval, P-wave duration, QRS complex duration and Bazett-corrected QT interval. Previously derived magnetic resonance imaging measurements for left ventricle[5] and thoracic aorta[108] were available for approximately 21,000 and 20,000 individuals, respectively. The exact breakdown of samples for each trait is presented in Supplementary Table 3. All performed tests were two-sided unless otherwise specified. To determine statistical significance, we applied a Benjamini-Hochberg false discovery rate (FDR) to compute Q-values from two-sided *P*-values across all performed tests (all genes for all traits combined). Tests with $Q < 0.01$ were considered significant. Associations with $0.01 \leq Q < 0.05$ were considered suggestive.

Sensitivity analyses for variant annotation and ancestry.

For all identified significant associations, we ran a number of sensitivity analyses. First, we restricted analyses to LOF variants only to evaluate the consistency of effect sizes for the combined set of LOFs and damaging missense variants as compared to LOFs only. Second, we restricted analyses to individuals of white-British European ancestry only, as determined by previous principal component analysis[63], to evaluate whether results were affected strongly by our multi-ancestry approach.

Leave-one-variant-out analysis.

To assess the robustness of our gene-based results to changes in the variant mask, we performed leave-one-variant-out (LOVO) analyses. For all significant associations, we iteratively reran the association test for each variant included in the original mask, after removing that variant from the mask. We defined the maximum LOVO *P*-value as the highest *P*-value attained for a given gene-phenotype association by this procedure. The variant that was removed to attain the maximum LOVO *P*-value was considered the most important variant for the gene-phenotype association.

Conditional analyses adjusting for nearby common variation.

To show that the identified rare variant signals were independent of nearby common variants, we reran the significant gene-phenotype associations while conditioning on common variants in the region. For a given gene-phenotype association, we first ran common variant (MAF > 0.5%) association analyses in the genomic region 500 kb downstream and upstream of the identified gene, using UK Biobank imputed data (Supplementary Note). We then clumped and thresholded the results to identify independent index common variants within the region using the *--clump* function in PLINK[109], utilizing cutoffs of $P < 1 \times 10^{-5}$ and $r^2 < 0.01$. Gene-based rare variant association analyses were then

rerun within individuals who had both exome sequencing and imputed data available, adding each of the clumped common variants to the model as fixed-effect covariates.

Replication of novel rare variant signals.

We sought to replicate novel rare variant associations within the Geisinger MyCode cohort. The MyCode Community Health Initiative is a research study of the Geisinger health system in central and northeastern Pennsylvania[21,110]. Started in 2007, the study is open to any Geisinger patient—through opt-in informed consent—including both primary and specialty care clinics, and has enrolled over 280,000 participants to date. Through the DiscovEHR collaboration with Regeneron Genetics Center, whole-exome sequencing from collected blood samples has been completed for approximately 175,000 participants to date, and linked with health information from the Geisinger electronic health record (1996–present). This study leveraged exome data for over 166,661 adult (≥18) individuals uniformly sequenced using an IDT exome capture platform and who passed subsequent central quality control procedures. The Geisinger Institutional Review Board approved the MyCode project and the present analysis.

Given the many parallel analyses of our discovery dataset, we define ‘novel’ to indicate rare variant associations not described prior to the release of the UK Biobank exome data. Using this definition, we identified 15 novel associations, of which 14 were testable in MyCode (those with adequate number of samples available). Gene-based collapsing tests were performed in this cohort, including rare LOF and predicted-deleterious missense variants, as described for the UK Biobank discovery analysis (except for the use of LOFTEE). We tested the 14 novel gene-phenotype associations using REGENIE[59], implementing a logistic whole-genome ridge regression model (which accounts for the relatedness among study samples) and further including age, sex, and associated PCs (1-4) as additional fixed-effects. The null model was fit using genome-wide genotype data (MAF > 0.05) from the same patients acquired on the Illumina GSA v2 chip. We additionally replicated the association between *PLIN1* and high-density lipoprotein, because the direction of effect for *PLIN1* LOF variants was different to the reported effect from small studies focused on partial lipodystrophy[37]. Therefore, in sum, we attempted replication for 15 rare variant associations in the MyCode cohort.

We further utilized the Type 2 Diabetes Knowledge Portal (T2DKP)[111] to seek direct replication of novel rare variant associations for type 2 diabetes. We focused on the dataset described in a previous large scale whole exome sequencing analysis of type 2 diabetes (n = 20,791 cases and 24,440 controls)[13], using the portal to look up results for rare LOF variants.

Pathogenic variation for cardiometabolic genes in the population.

Given the high prevalence of cardiometabolic disease, we then sought to quantify carrier frequencies for putatively pathogenic variation in cardiovascular genes in the population. We analyzed genes included on typical sequencing panels for Mendelian cardiovascular disease, namely the *Invitae Arrhythmia and Cardiomyopathy* panel and the *Invitae Hypercholesterolemia* panel (accessed on 10 November 2020). We then restricted to genes

reported for autosomal dominant modes of inheritance in the *Online Mendelian Inheritance in Man* (OMIM) database (accessed on 10 November 2020). Using ClinVar, we identified carriers of rare (MAF < 0.1% in the exome sequencing dataset and MAF < 0.1% in the continental gnomAD populations) pathogenic or likely pathogenic variants in each genes in the UK Biobank (Supplementary Note). We collapsed carrier status for LOFs (affecting canonical gene transcripts), pathogenic and likely pathogenic variants for each gene, with a few exceptions: for *TTN*, we restricted to LOF variants located in exons highly expressed in cardiac tissue[112] (Supplementary Note); for *RYR2*, *PCSK9*, *APOB*, *MYH7* and *TTR*, analyses were restricted to pathogenic and likely pathogenic variants only given well-characterized gain-of-function or non-truncating mechanisms causing dominant cardiovascular disease[113-116]. After collapsing as described, individuals harboring these variants were considered carriers of putatively pathogenic rare variants. Next, we calculated the percentage of the study sample that carried putatively pathogenic variants in each gene.

We also analyzed monogenic diabetes genes in a similar manner. We used genes included on the *Invitae Monogenic Diabetes* panel that were reported in the OMIM database to be associated with autosomal dominant forms of type 2 diabetes, insulin-dependent diabetes, or Maturity-Onset Diabetes of the Young. Again, we collapsed carrier status for LOF, pathogenic and likely pathogenic variants for each included gene, with a few exceptions. For *ABCC8* and *KCNJ11*, we restricted to pathogenic and likely pathogenic variants only, based on known gain-of-function mechanisms causing dominant diabetes[117,118].

We then employed the same logistic mixed-model approach described above to identify associations between putatively pathogenic variants in genes and a range of relevant diseases and outcomes (20 diseases for the cardiovascular analysis and 3 diseases for the diabetes analysis). Significance was determined using a separate FDR correction, taking into account all tests performed for the cardiovascular and diabetes analysis combined. Associations at Q < 0.01 were considered significant. Firth's regression was used to estimate ORs and CIs in the unrelated subset of the sample.

Data Availability

Summary results for the main analyses have been made available through the Cardiovascular Disease Knowledge Portal (https://cvd.hugeamp.org/downloads.html; direct download using https://personal.broadinstitute.org/ryank/Ellinor_ukbb_200k_exome.zip). Access to individual-level UK Biobank data, both phenotypic and genetic, is available to bona fide researchers through application on the UK Biobank website (https://www.ukbiobank.ac.uk). The exome sequencing data can be found in the UK Biobank showcase portal https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=170. Additional information about registration for access to the data is available at http://www.ukbiobank.ac.uk/register-apply/. Use of UK Biobank data was performed under application number 17488.

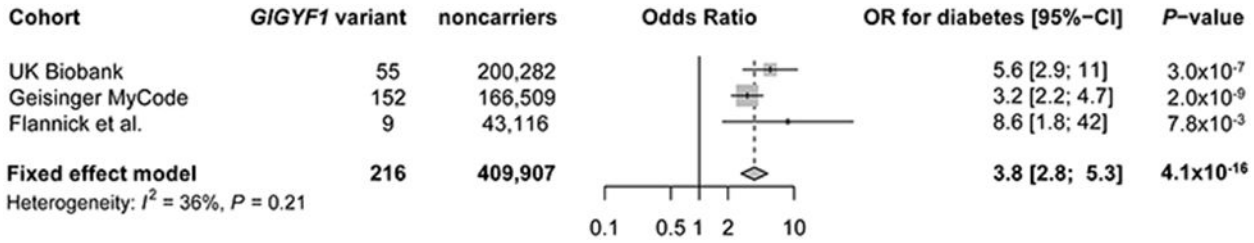
Summary statistics from previous GWAS which were utilized in this study are publicly available through the Type 2 Diabetes Knowledge Portal (https://t2d.hugeamp.org); MAGMA results referenced in this manuscript were downloaded on 7 December 2020, while index single variant results were downloaded on 7 June 2021.

Other datasets utilized in this manuscript include: the dbNSFP database version 4.1a (https://sites.google.com/site/jpopgen/dbNSFP); gnomAD exomes version 2.1 (https://gnomad.broadinstitute.org/downloads); the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) downloaded in November 2020; the Invitae Arrhythmia and Cardiomyopathy panel (https://www.invitae.com/en/physician/tests/02101/) and the Invitae Hypercholesterolemia panel (https://www.invitae.com/en/physician/tests/02401/) accessed on 10 November 2020; the Invitae Monogenic Diabetes panel (https://www.invitae.com/pt/physician/tests/55001/) accessed in January 2021; the Online Mendelian Inheritance in Man (OMIM) database (omim.org) accessed on 10 November 2020; Ensembl release 95 (https://gnomad.broadinstitute.org/downloads); and the GTEx dataset version 8 (https://gtexportal.org/home/).

Code Availability

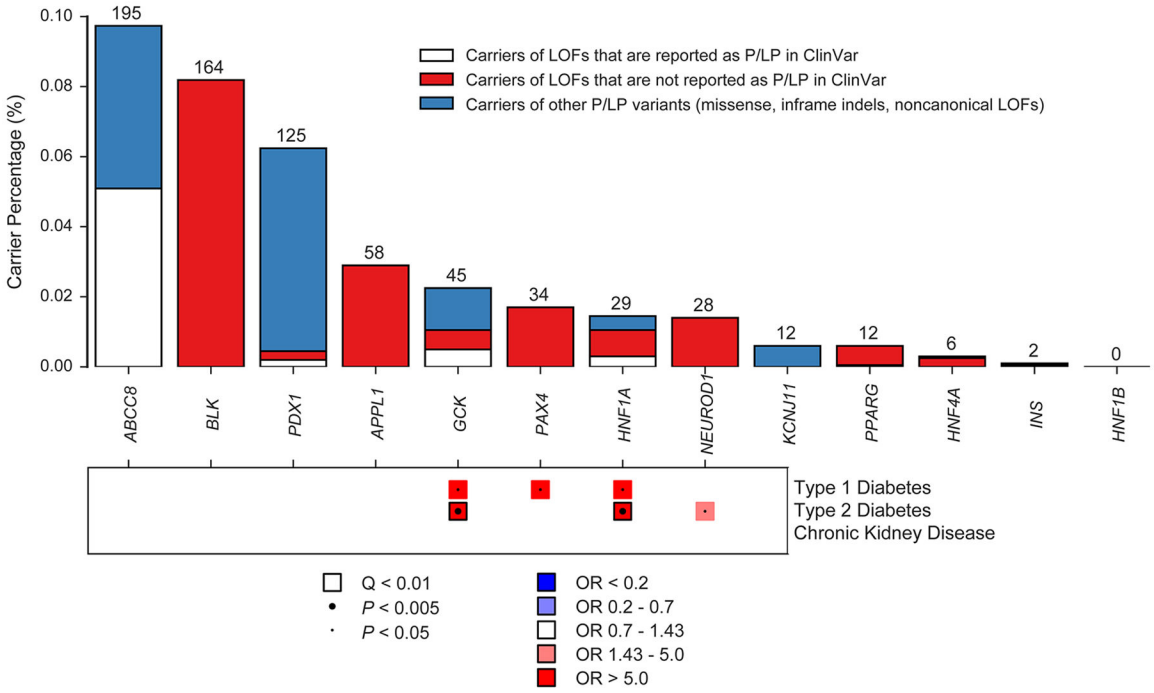
The code used for gene-based analyses is an adaptation of the R package GENESIS version 2.18 (https://rdrr.io/bioc/GENESIS/man/GENESIS-package.html), and has been made available through the following GitHub repository: https://github.com/seanjosephjurgens/UKBB_200KWES_CVD. Quality-control of individual level data was performed using Hail version 0.2 (https://hail.is), PLINK version 2.0.a (https://www.cog-genomics.org/plink/2.0/), and KING version 2.2.5 (https://www.kingrelatedness.com/Download.shtml). Variant annotation was performed using VEP version 95 (https://github.com/Ensembl/ensembl-vep) with the LOFTEE plug-in (https://github.com/konradjk/loftee). All analyses that were run in R were run in R version 4.0 (https://www.r-project.org).

Extended Data



Extended Data Fig. 1. Meta-analysis results for *GIGYF1* rare variants and type 2 diabetes across three cohorts

Data are presented in a forest plot, with study specific odds ratios (OR) with 95% confidence intervals (95% CI), and a meta-analysis OR shown with a diamond where the edges of the diamond show the meta-analysis 95% CI. Meta-analysis results are obtained from an inverse-variance weighted fixed-effects meta-analysis approach. Study-specific and meta-analysis *P*-values are two-sided and unadjusted for multiple testing. To evaluate heterogeneity between studies, an I^2 index for heterogeneity and a *P*-value from Cochran's Q test are provided, which show limited evidence of heterogeneity.



Extended Data Fig. 2. Carrier frequencies of putatively pathogenic variants in monogenic diabetes genes

The top of the graph is a bar chart showing carrier frequencies for loss-of-function (LOF) variants and pathogenic/likely pathogenic (P/LP) variants for genes in which variants are known to cause dominant type 2 diabetes or maturity-onset diabetes of the young (MODY). For *ABCC8* and *KCNJ11*, analyses were restricted to previously reported P/LP variants only. The bottom of the graph is a pruned heatmap showing associations between such variants with diabetes and chronic kidney disease, where blue indicates lower risk of disease and red indicates increased risk of the disease. *P*-values were computed using saddle point approximation and were obtained from logistic mixed effects models, adjusting for sex, age, sequencing batch, associated principal components (PCs), a sparse kinship matrix. *P*-values shown are two-sided and unadjusted for multiple testing. Odds ratios (OR) were obtained from Firth's regression models adjusting for sex, age, sequencing batch and associated PCs among unrelated samples. For clarity, associations with $P > 0.05$ and $0.7 < \text{OR} < 1.43$ have been made white. Only *GCK* (45 carriers) and *HNF1A* (29 carriers) showed robust associations with diabetes. Of note, *PDX1* carriers are driven by a single likely pathogenic missense variant, p.Cys18Arg ($n = 112$ carriers). Our results therefore indicate that this allele specifically does not represent a highly penetrant pathogenic variant, but do not necessarily translate to the 13 carriers of LOF variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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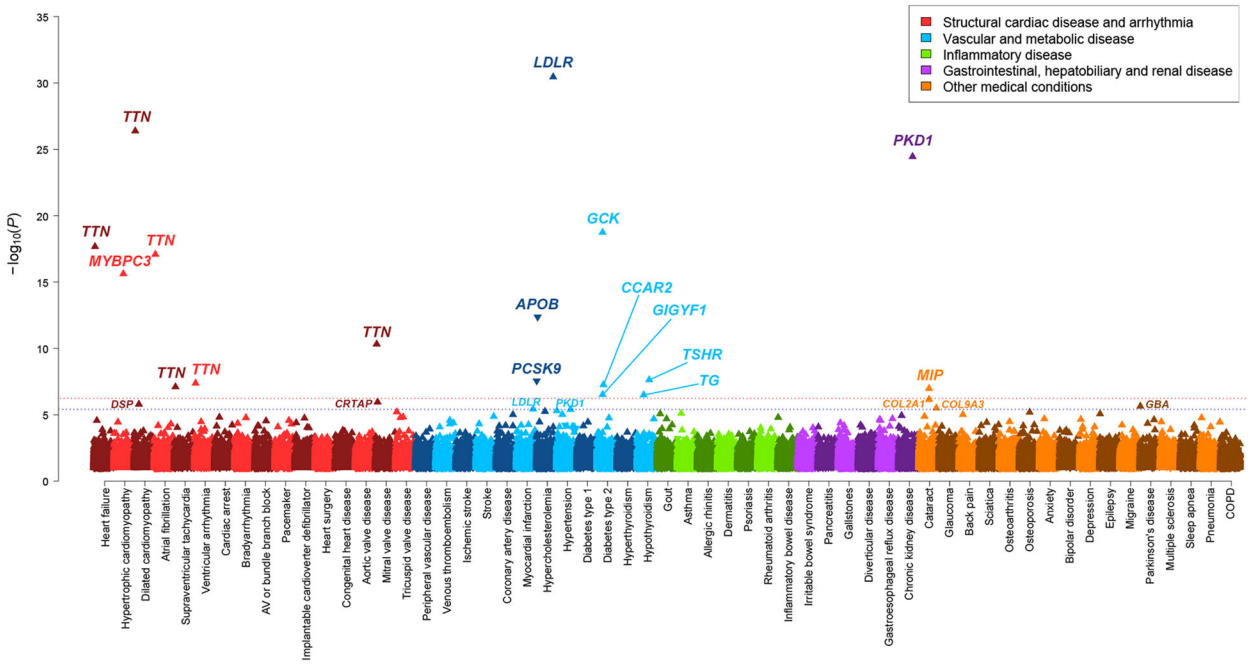

Figure 1 |. Rare genetic variation for 57 cardiometabolic and other medical disorders in the UK Biobank.

Multiple-trait Manhattan plot representing the results from exome-wide gene-based tests for each phenotype. Phenotypes are labelled on the *x*-axis and the $-\log_{10}$ of the *P*-value for each test on the *y*-axis. Variants included in the gene-based test are restricted to loss-of-function and predicted-deleterious missense variants. *P*-values were computed using the saddle point approximation and were obtained from logistic mixed effects models, adjusting for sex, age, sequencing batch, associated principal components (PCs) and a sparse kinship matrix. *P*-values shown are two-sided and unadjusted for multiple testing. The red line indicates the significance threshold at a Benjamini-Hochberg false-discovery-rate (FDR) of 1% across all tests across all binary and quantitative traits, while the blue line represents the suggestive threshold at FDR 5%. An arrow pointing upwards indicates that rare variants were associated with increased risk of disease, while arrows pointing downward indicate decreased risk.

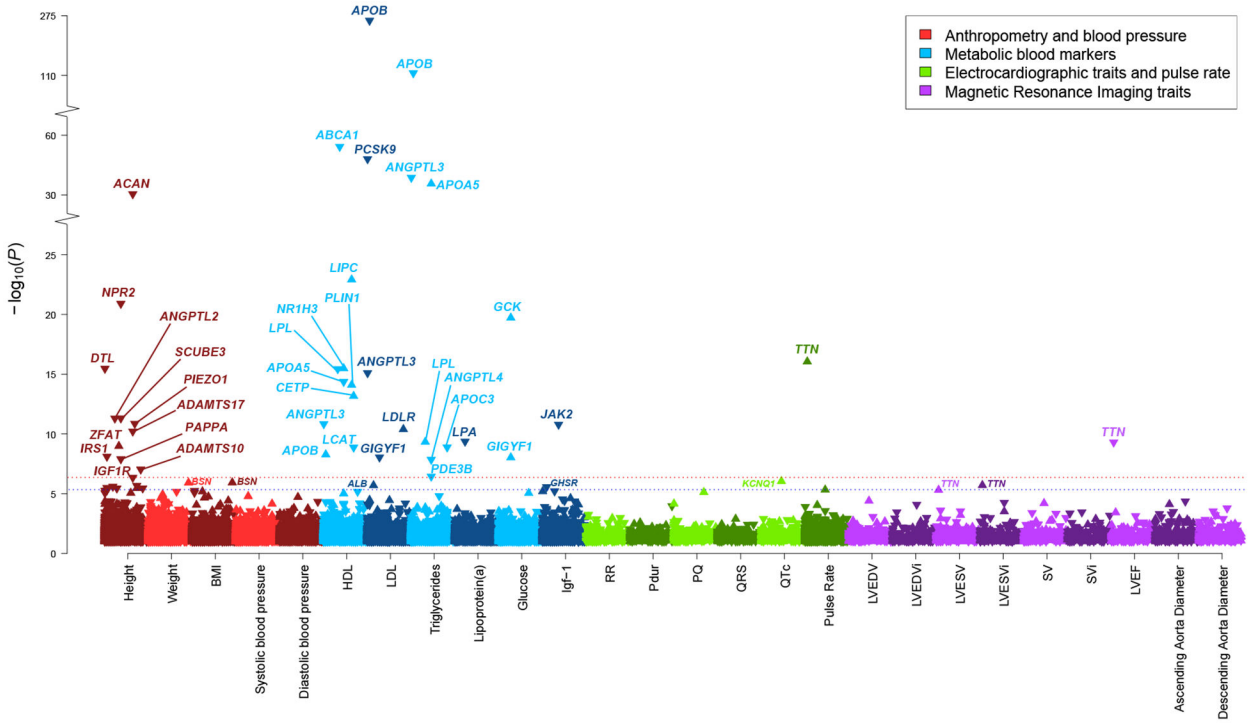

Figure 2 |. Rare genetic variation for 26 quantitative cardiometabolic traits in the UK Biobank. Multiple-trait Manhattan plot representing the results from exome-wide gene-based tests for each phenotype. Phenotypes are labelled on the *x*-axis and the $-\log_{10}$ of the *P*-value for each test on the *y*-axis. Variants included in the gene-based test are restricted to loss-of-function and predicted-deleterious missense variants. *P*-values were obtained from score tests from linear mixed effects models, adjusting for sex, age, sequencing batch, associated principal components (PCs), MRI serial number (for MRI traits) and a sparse kinship matrix. *P*-values shown are two-sided and unadjusted for multiple testing. The red line indicates the significance threshold at a Benjamini-Hochberg false-discovery rate (FDR) of 1% across all tests across all binary and quantitative traits, while the blue line represents the suggestive threshold at FDR 5%. For height, suggestively associated genes are not annotated with gene names for clarity. An arrow pointing upwards indicates that rare variants were associated with higher value for a given quantitative trait, while arrows pointing downward indicate lower value. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Igf-1, insulin-like growth factor 1; RR, RR interval; Pdur, P-wave duration; PQ, PQ interval; QRS, QRS-complex duration; QTc, Bazett-corrected QT interval; LVEDV, left ventricular end-diastolic volume; LVEDVi, body-surface-area indexed left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVESVi, body-surface-area indexed left ventricular end-systolic volume; SV, stroke volume; SVi, body-surface-area indexed stroke volume; LVEF, left ventricular ejection fraction.

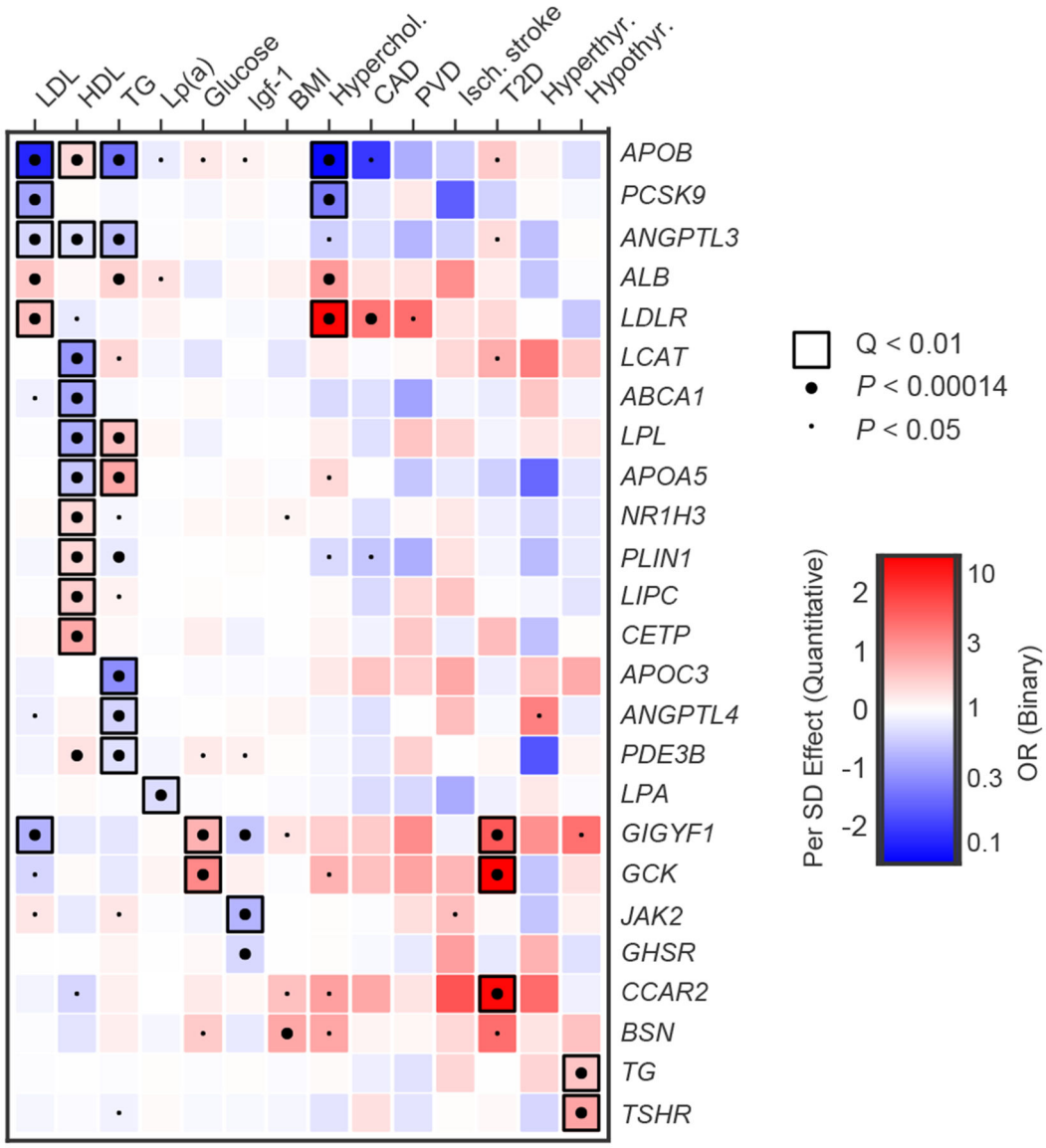

Figure 3 |. Pleiotropy of rare variants in metabolic genes.

This heatmap shows association results for genes associated at false-discovery rate (FDR) Q-value < 0.05 with any metabolic trait in our primary analysis, across a range of relevant metabolic traits. *P*-values were obtained from score tests in linear mixed effects models (quantitative traits) or saddle point approximation in logistic mixed effects models (binary traits), adjusting for sex, age, sequencing batch, associated principal components (PCs) and a sparse kinship matrix. *P*-values shown are two-sided and unadjusted for multiple testing, while Q-values represent false-discovery rate (FDR) adjusted two-sided *P*-values by Benjamini-Hochberg method. Effect sizes for quantitative traits (β) were obtained from the same linear model, while odds ratios (OR) for binary traits were obtained from Firth's regression models adjusting for sex, age, sequencing batch and associated PCs among unrelated samples. A small dot indicates nominal significance ($P < 0.05$), a large dot indicates $P < 0.00014$ (0.05/ 350 tests), while a black square indicates FDR Q-value <

0.01 in the primary discovery phase. Red indicates $\beta > 0$ (quantitative traits) or OR > 1 (binary traits), while blue indicates $\beta < 0$ (quantitative traits) or OR < 1 (binary traits). LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; Lp(a), lipoprotein (a); Igf-1, insulin-like growth factor 1; Hyperchol, hypercholesterolemia; CAD, coronary artery disease; PVD, peripheral vascular disease; Isch. Stroke, ischemic stroke; T2D, type 2 diabetes.

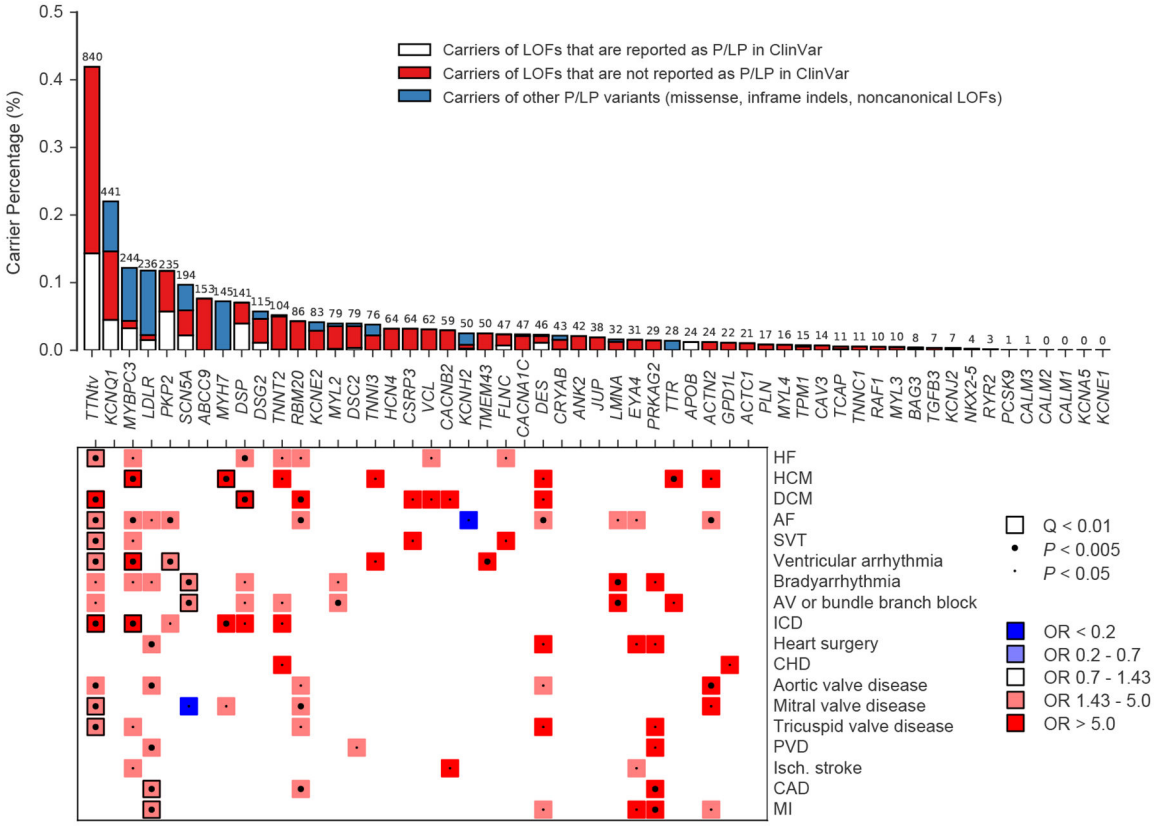

Figure 4 |. Putatively pathogenic variants in Mendelian cardiovascular disease genes in the UK Biobank.

The top of the figure is a bar chart showing carrier frequencies for rare LOF, pathogenic and likely pathogenic variants in genes reported for dominant inheritance of arrhythmia, cardiomyopathy or hypercholesterolemia. The absolute number of carriers in the UK Biobank is shown above each bar. Bar charts are stacked to visualize carriers of LOFs reported in ClinVar as likely pathogenic or pathogenic (P/LP), carriers of LOFs not reported in ClinVar as P/LP, and carriers of other P/LP variants (missense, inframe indels, noncanonical or low-confidence LOFs, etc). The bottom of the figure is a pruned heatmap showing association results between these variants and cardiovascular outcomes that reach nominal significance ($P < 0.05$). *P*-values were computed using the saddle point approximation and were obtained from logistic mixed effects models, adjusting for sex, age, sequencing batch, associated principal components (PCs) and a sparse kinship matrix. *P*-values shown are two-sided and unadjusted for multiple testing. Odds ratios (OR) were obtained from Firth's regression models adjusting for sex, age, sequencing batch and associated PCs among unrelated samples. A small dot represents nominal significance ($P < 0.05$), while a large dot represents $P < 0.005$. A square represents significant at an FDR Q-value of < 0.01. Blue indicates OR < 1, while red indicates OR > 1. For clarity, tests with $P > 0.05$ or an OR between 0.7 and 1.43 have been made white. HF, heart failure; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; AF, atrial fibrillation; SVT, supraventricular tachycardia; AV, atrioventricular; ICD, implantable

cardioverter-defibrillator; CHD, congenital heart disease; PVD, peripheral vascular disease; Isch., Ischemic; CAD, coronary artery disease; MI, myocardial infarction.

Table 1 |

Baseline characteristics of participants in the UK Biobank with exome sequencing data

Participants, *n*	200,337
Female, *n* (%)	110,359 (55.1)
European ancestry, *n* (%)	174,879 (87.3)
Age at baseline, mean (s.d.)	56.97 (8.10)
Age at last follow-up, mean (s.d.)	67.82 (8.07)
Height in cm, mean (s.d.)	168.47 (9.27)
Body mass index, median (IQR)	26.70 (5.72)
Cardiovascular and metabolic diseases	
Atrial fibrillation, *n* (%)	12,277 (6.1)
Supraventricular arrhythmia, *n* (%)	2,075 (1.0)
Ventricular arrhythmia, *n* (%)	2,072 (1.0)
Mitral valve disease, *n* (%)	3,898 (1.9)
Hypertension, *n* (%)	74,347 (37.1)
Heart failure, *n* (%)	5,344 (2.7)
Dilated cardiomyopathy, *n* (%)	377 (0.2)
Hypertrophic cardiomyopathy, *n* (%)	220 (0.1)
Myocardial Infarction, *n* (%)	6,238 (3.1)
Hypercholesterolemia, *n* (%)	42,799 (21.4)
Diabetes type 2, *n* (%)	14,607 (7.3)
Hypothyroidism, *n* (%)	14,097 (7.0)
Malignancy	
Breast cancer, *n* (%)	8,112 (7.4)
Colorectal cancer, *n* (%)	2,733 (1.4)
Other medical conditions	
Chronic kidney disease, *n* (%)	6,415 (3.2)
Cataracts, *n* (%)	21,762 (10.9)

Values are presented as number (percentage) unless otherwise specified. s.d., standard deviation; IQR, interquartile range.

Table 2 |

Gene associations with cardiometabolic and other diseases at FDR Q-value < 0.01

Trait	Gene	Carriers	Cases among carriers (%)	Cases among noncarriers (%)	OR [95% CI]	*P*-value	Ref
Known associations							
Hypertrophic cardiomyopathy	*MYBPC3*	93	9 (9.68)	211 (0.11)	120.38 [55.94-231.86]	2.39×10^{-16}	65,66
Heart failure	*TTN*	1,858	121 (6.51)	5,223 (2.63)	2.64 [2.15-3.20]	2.14×10^{-18}	67,68
Dilated cardiomyopathy	*TTN*	1,741	38 (2.11)	339 (0.17)	12.20 [8.44-17.09]	4.14×10^{-27}	68
Atrial fibrillation	*TTN*	1,858	211 (11.36)	12,066 (6.08)	2.06 [1.75-2.40]	8.23×10^{-18}	19,57,69
Ventricular arrhythmia	*TTN*	1,858	47 (2.53)	2,025 (1.02)	2.45 [1.77-3.30]	4.18×10^{-8}	28
Diabetes type 2	*GCK*	64	31 (48.44)	14,576 (7.28)	13.98 [8.33-23.42]	1.80×10^{-19}	70
Chronic kidney disease	*PKD1*	51	24 (47.06)	6,391 (3.19)	40.33 [21.27-76.24]	3.54×10^{-25}	71-73
Hypercholesterolemia	*LDLR*	104	74 (71.15)	42,725 (21.34)	13.11 [8.28-21.26]	3.53×10^{-31}	74-76
	APOB	247	6 (2.43)	42,793 (21.39)	0.08 [0.03-0.18]	4.27×10^{-13}	77
	PCSK9	258	20 (7.75)	42,779 (21.38)	0.26 [0.15-0.42]	2.78×10^{-8}	78
Hypothyroidism	*TSHR*	304	48 (15.79)	14,049 (7.02)	2.53 [1.79-3.49]	2.34×10^{-8}	79
	TG	785	97 (12.36)	14,000 (7.02)	1.83 [1.45-2.28]	3.18×10^{-7}	80,81
Novel associations *							
Diabetes type 2	*GIGYF1*	55	16 (29.09)	14,591 (7.29)	5.61 [2.90-10.32]	3.04×10^{-7}	
	CCAR2	26	11 (42.31)	14,596 (7.29)	12.79 [5.63-28.44]	5.43×10^{-8}	
Supraventricular tachycardia	*TTN*	1,858	46 (2.48)	2,029 (1.02)	2.40 [1.73-3.23]	7.88×10^{-8}	
Mitral valve disease	*TTN*	1,858	81 (4.36)	3,817 (1.92)	2.31 [1.80-2.91]	4.74×10^{-11}	

P-values were computed using the saddle point approximation and were obtained from logistic mixed effects models, adjusting for sex, age, sequencing batch, associated principal components (PCs) and a sparse kinship matrix. *P*-values shown are unadjusted for multiple testing. Odds ratios (OR) and 95% confidence intervals (95% CI) were obtained from Firth's regression models adjusting for sex, age, sequencing batch and associated PCs among unrelated individuals.

*Novel indicates that rare variant associations were not reported prior to the release of UK Biobank exomes. Ref, references.

Table 3 |

Gene associations for quantitative cardiometabolic traits at FDR Q-value < 0.01

Trait	Gene	Carriers	Effect in s.d. [95% CI]	*P*-value	Ref
Known associations					
Height, cm	*ADAMTS17*	173	−0.34 [−0.45, −0.24]	6.14×10^{-11}	82,83
	ACAN	42	−1.17 [−1.38, −0.96]	6.86×10^{-28}	84
	NPR2	114	−0.62 [−0.75, −0.49]	1.26×10^{-21}	85-87
	IGF1R	51	−0.49 [−0.68, −0.30]	4.56×10^{-7}	88
	ADAMTS10	24	−0.76 [−1.03, −0.48]	9.35×10^{-8}	89,90
Glucose	*GCK*	56	1.22 [0.96, 1.48]	1.91×10^{-20}	91
HDL	*ABCA1*	236	−0.92 [−1.03, −0.80]	5.90×10^{-55}	92
	APOA5	156	−0.57 [−0.71, −0.43]	4.30×10^{-15}	12
	ANGPTL3	310	−0.35 [−0.45, −0.25]	1.47×10^{-11}	93
	PLIN1	315	0.40 [0.30, 0.49]	8.01×10^{-15}	37
	LCAT	27	−1.05 [−1.39, −0.71]	1.34×10^{-9}	94
	LPL	78	−0.83 [−1.03, −0.63]	3.68×10^{-16}	92
	LIPC	320	0.51 [0.41, 0.60]	1.23×10^{-23}	95
	APOB	201	0.37 [0.25, 0.50]	5.28×10^{-9}	96
	CETP	58	0.89 [0.66, 1.12]	6.80×10^{-14}	97
LDL	*PCSK9*	245	−0.94 [−1.06, −0.81]	9.19×10^{-49}	16
	ANGPTL3	363	−0.42 [−0.52, −0.32]	7.98×10^{-16}	93
	APOB	237	−2.24 [−2.37, −2.11]	2.94×10^{-262}	98
	LDLR	98	0.66 [0.47, 0.86]	4.06×10^{-11}	99
Triglycerides	*APOA5*	180	0.91 [0.77, 1.05]	2.31×10^{-36}	12,100
	ANGPTL3	363	−0.67 [−0.77, −0.57]	2.03×10^{-39}	93
	ANGPTL4	173	−0.45 [−0.59, −0.30]	1.32×10^{-9}	35
	APOB	237	−1.45 [−1.57, −1.32]	3.21×10^{-117}	77
	LPL	86	0.65 [0.45, 0.85]	4.68×10^{-10}	100
	APOC3	22	−1.17 [−1.58, −0.77]	1.42×10^{-8}	101
	PDE3B	224	−0.33 [−0.46, −0.20]	3.63×10^{-7}	4
Lipoprotein (a)	*LPA*	307	−0.35 [−0.46, −0.24]	4.30×10^{-10}	102
LVEF	*TTN*	179	−0.44 [−0.57, −0.30]	5.01×10^{-10}	56,64
Pulse rate	*TTN*	1,707	0.20 [0.15, 0.25]	8.82×10^{-17}	19
Novel associations *					
Height, cm	*SCUBE3*	71	−0.57 [−0.73, −0.41]	5.02×10^{-12}	
	PIEZO1	574	−0.20 [−0.25, −0.14]	1.39×10^{-11}	
	IRS1	47	−0.58 [−0.78, −0.38]	7.81×10^{-9}	
	ANGPTL2	119	−0.44 [−0.57, −0.32]	4.88×10^{-12}	

Trait	Gene	Carriers	Effect in s.d. [95% CI]	*P*-value	Ref
	PAPPA	36	−0.66 [−0.89, −0.43]	1.28×10^{-8}	
	ZFAT	53	0.58 [0.40, 0.77]	1.04×10^{-9}	
	DTL	72	−0.67 [−0.83, −0.51]	3.42×10^{-16}	
Igf-1	*JAK2*	70	−0.77 [−1.00, −0.55]	1.64×10^{-11}	
Glucose	*GIGYF1*	51	0.79 [0.52, 1.06]	9.45×10^{-9}	
HDL	*NR1H3*	352	0.39 [0.30, 0.49]	3.27×10^{-16}	
LDL	*GIGYF1*	52	−0.79 [−1.06, −0.52]	9.02×10^{-9}	

P-values, effect sizes and 95% confidence intervals (95% CI) were obtained from score tests from linear mixed effects models, adjusting for sex, age, sequencing batch, associated principal components (PCs), MRI serial number (for MRI traits) and a sparse kinship matrix. *P*-values shown are unadjusted for multiple testing.

*Novel indicates that rare variant associations were not reported prior to the release of UK Biobank exomes. s.d., standard deviation; Ref, reference; LVEF, left ventricular ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Igf-1, insulin-like growth factor 1.

Table 4 |

Replication of novel associations in the Geisinger MyCode cohort

Binary traits				
Trait	**Gene**	**Carriers**	**OR [95% CI]**	***P*-value**
Diabetes type 2	*GIGYF1*	152	3.18 [2.22, 4.54]	1.98×10^{-9}
	CCAR2	24	0.75 [0.24, 2.33]	0.62
Supraventricular tachycardia	*TTN*	3,059	1.35 [1.08, 1.70]	0.01
Mitral valve disease	*TTN*	3,059	1.45 [1.18, 1.78]	8.26×10^{-4}
Quantitative traits				
Trait	**Gene**	**Carriers**	**Effect in s.d. [95% CI]**	***P*-value**
Height, cm	*SCUBE3*	153	−0.67 [−0.95, −0.39]	3.90×10^{-6}
	PIEZO1	577	−0.35 [−0.50, −0.21]	1.97×10^{-6}
	IRS1	36	−0.67 [−1.24, −0.11]	0.02
	ANGPTL2	78	−1.02 [−1.41, −0.64]	2.45×10^{-7}
	PAPPA	69	−0.47 [−0.89, −0.05]	0.03
	ZFAT	42	0.79 [0.24, 1.34]	5.04×10^{-3}
	DTL	45	−0.90 [−1.41, −0.39]	5.69×10^{-4}
Glucose	*GIGYF1*	102	0.55 [0.37, 0.73]	1.37×10^{-9}
HDL	*NR1H3*	122	0.38 [0.22, 0.54]	2.97×10^{-6}
	PLIN1 *	97	0.36 [0.18, 0.54]	9.29×10^{-5}
LDL	*GIGYF1*	105	−0.29 [−0.47, −0.11]	1.76×10^{-3}

P-values, odds ratios (OR) and 95% confidence intervals (95% CI) were obtained from whole-genome ridge-regression models implemented in REGENIE, further adjusting for sex, age and associated principal components (PCs). *P*-values shown are unadjusted for multiple testing. Novel indicates that rare variant associations were not reported prior to the release of UK Biobank exomes. Association test between Igf-1 and *JAK2* was not performed due to low cumulative minor allele counts (cMAC); cMAC among individuals with Igf-1 was 2.

* The association between *PLIN1* and HDL was added to replication because the direction of effect (increased HDL) was different to the direction reported in family-based studies of partial lipodystrophy.