Title

Rare Coding Variants Associated With Electrocardiographic Intervals Identify Monogenic Arrhythmia Susceptibility Genes: A Multi-Ancestry Analysis.

Journal

Circulation. Genomic and precision medicine, 14(4)

ISSN

2574-8300

Authors

Choi, Seung Hoan
Jurgens, Sean J
Haggerty, Christopher M
et al.

Publication Date

2021-08-01

DOI

10.1161/circgen.120.003300

Peer reviewed

Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Rare Coding Variants Associated With Electrocardiographic Intervals Identify Monogenic Arrhythmia Susceptibility Genes

A Multi-Ancestry Analysis

Seung Hoan Choi, PhD*; Sean J. Jurgens, BSc*; Christopher M. Haggerty, PhD; Amelia W. Hall, PhD; Jennifer L. Halford, MD; Valerie N. Morrill, MS; Lu-Chen Weng, PhD; Braxton Lagerman, BS; Tooraj Mirshahi, PhD; Mary Pettinger, MS; Xiuqing Guo, PhD; Henry J. Lin, MD; Alvaro Alonso, MD, PhD; Elsayed Z. Soliman, MD, MSc, MS; Jelena Kornej, MD, MSc; Honghuang Lin, PhD; Arden Moscati, PhD; Girish N. Nadkarni, MD; Jennifer A. Brody, BA; Kerri L. Wiggins, MS; Brian E. Cade, PhD; Jiwon Lee, MS; Christina Austin-Tse, PhD; Tom Blackwell, PhD; Mark D. Chaffin, MS; Christina J.-Y. Lee, MD, PhD; Heidi L. Rehm, PhD; Carolina Roselli, MS; Regeneron Genetics Center; Susan Redline, MD, MPH; Braxton D. Mitchell, PhD, MPH; Nona Sotoodehnia, MD, MPH; Bruce M. Psaty, MD, PhD; Susan R. Heckbert, MD, PhD; Ruth J.F. Loos, PhD; Ramachandran S. Vasan, MD; Emelia J. Benjamin, MD, ScM; Adolfo Correa, MD, PhD; Eric Boerwinkle, PhD; Dan E. Arking, PhD; Jerome I. Rotter, MD; Stephen S. Rich, PhD; Eric A. Whitsel, MD, MPH; Marco Perez, MD; Charles Kooperberg, PhD; Brandon K. Fornwalt, MD, PhD; Kathryn L. Lunetta, PhD; Patrick T. Ellinor, MD, PhD†; Steven A. Lubitz, MD, MPH†; on behalf of the NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium

BACKGROUND: Alterations in electrocardiographic (ECG) intervals are well-known markers for arrhythmia and sudden cardiac death (SCD) risk. While the genetics of arrhythmia syndromes have been studied, relations between electrocardiographic intervals and rare genetic variation at a population level are poorly understood.

METHODS: Using a discovery sample of 29 000 individuals with whole-genome sequencing from Trans-Omics in Precision Medicine and replication in nearly 100 000 with whole-exome sequencing from the UK Biobank and MyCode, we examined associations between low-frequency and rare coding variants with 5 routinely measured electrocardiographic traits (RR, P-wave, PR, and QRS intervals and corrected QT interval).

RESULTS: We found that rare variants associated with population-based electrocardiographic intervals identify established monogenic SCD genes (*KCNQ1*, *KCNH2*, and *SCN5A*), a controversial monogenic SCD gene (*KCNE1*), and novel genes (*PAM* and *MFGE8*) involved in cardiac conduction. Loss-of-function and pathogenic *SCN5A* variants, carried by 0.1% of individuals, were associated with a nearly 6-fold increased odds of the first-degree atrioventricular block ($P=8.4\times10^{-5}$). Similar variants in *KCNQ1* and *KCNH2* (0.2% of individuals) were associated with a 23-fold increased odds of marked corrected QT interval prolongation ($P=4\times10^{-25}$), a marker of SCD risk. Incomplete penetrance of such deleterious variation was common as over 70% of carriers had normal electrocardiographic intervals.

CONCLUSIONS: Our findings indicate that large-scale high-depth sequence data and electrocardiographic analysis identifies monogenic arrhythmia susceptibility genes and rare variants with large effects. Known pathogenic variation in conventional arrhythmia and SCD genes exhibited incomplete penetrance and accounted for only a small fraction of marked electrocardiographic interval prolongation.

Key Words: death, sudden, cardiac ■ epidemiology ■ genetics ■ genome ■ population

Correspondence to: Steven A. Lubitz, MD, MPH, Cardiac Arrhythmia Service and Cardiovascular Research Center, Massachusetts General Hospital, 185 Cambridge St, 3.188, Boston, MA 02114. Email slubitz@mgh.harvard.edu

*S.H. Choi and S.J. Jurgens are joint first authors.

†P.T. Ellinor and S.A. Lubitz are joint supervisors.

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.120.003300.

For Sources of Funding and Disclosures, see page 472, 473.

Nonstandard Abbreviation and Acronyms

LOF	loss-of-function
LQTS	long QT syndrome
QTc	corrected QT interval
SCD	sudden cardiac death
TOPMed	Trans-Omics in Precision Medicine

Nearly 1 in 3 adults will experience an arrhythmia and up to 10% will die of sudden cardiac death (SCD) during their lifetime.[1,2] The ECG is an inexpensive, noninvasive, and widely used screening test for abnormalities in cardiac conduction. Previous work has demonstrated that electrocardiographic intervals are quantitative markers for arrhythmias and SCD[3–8] and have a considerable heritable basis.[9]

Monogenic mutations underlying many conduction disorders and arrhythmia syndromes, such as the long QT syndrome (LQTS),[10,11] have been the focus of extensive study over the past 2 decades. Typically, these studies have focused on sequencing a modest number of affected patients or families to identify the causative genes. In contrast, large-scale genome-wide association studies have identified a multitude of loci associated with electrocardiographic traits by studying common variants in large study samples comprising thousands of individuals.[12–15] However, the effect of discovered genome-wide association studies variants is inherently small and direct implication of any particular gene tagged by an identified common variant is difficult.

To date, a missing gap in our understanding of common electrocardiographic traits and related diseases has been whether rare coding variants have a substantial contribution to variation at the population level. Rare variants may have substantial effect sizes, confer pathogenicity, and have a measurable impact on disease risk. Yet, identifying such variants has been technically challenging since this analysis requires both the sequencing of large study samples and the availability of electrocardiographic data.

To address this challenge, we used a unique resource of over 130 000 individuals with whole-exome or -genome sequencing, and a rigorous 3-stage design, to examine the associations between rare coding genetic variation and several routinely collected electrocardiographic traits, including the heart rate, maximum P-wave duration, PR interval, QRS duration, and corrected QT interval (QTc). We then specifically assessed the frequency, magnitude of association, and penetrance of clinically pathogenic variation in select SCD genes that are associated with electrocardiographic variation.

METHODS

Detailed methods are provided in the Data Supplement. The data that support the findings of this study are available from the corresponding author upon reasonable request. There are restrictions to the availability of raw Trans-Omics in Precision Medicine (TOPMed) and MyCode phenotypic and genotypic data due to the identifiable nature of this data. UK Biobank raw data are available to researchers via application through the UK Biobank website. All TOPMed participants provided written informed consent, and participating studies obtained ethical approval from their local institutional review boards. 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 Brigham Institutional Review Board.

RESULTS

An overview of the study design and sample selection flow is presented in Figure 1 and Figures I through III in the Data Supplement. Characteristics of the included studies are displayed in the Table and Tables I through III and Figure IV in the Data Supplement.

Once we had aggregated electrocardiographic and genetic data for participants in TOPMed, we began by performing genome-wide association studies for common variants related to 5 electrocardiographic traits (Figure 2A and Table IV in the Data Supplement). As expected, we observed associations at 44 previously reported loci (Results in the Data Supplement). Cross-trait pleiotropy was particularly notable at the *SCN5A-10A* locus and the *CAV1* locus, which demonstrated robust associations across multiple electrocardiographic traits.

We then performed an analysis of the protein-coding regions of the genome and identified a low-frequency coding variant in *PAM* that was associated with PR interval duration (p.Ser539Trp, rs78408340_G, MAF=0.5%, β=8 ms, $P=1.9\times10^{-7}$, Figure 2B and Table V in the Data Supplement). This finding was replicated in the UK Biobank (β=2 ms, $P=0.01$) and in MyCode (β=3 ms, $P=1.8\times10^{-4}$). Variants in *PAM* were also associated with PR interval duration in gene-based testing in TOPMed ($P=4.5\times10^{-7}$, Figure 3, Table VI in the Data Supplement), an association driven by p.Ser539Trp (Figure V in the Data Supplement). We did not observe consistent associations between rare high-confidence loss-of-function (LOF) variants in *PAM* and PR interval duration across data sets (Results, Tables VII and VIII, and Figure VIA in the Data Supplement). Low-frequency coding variant testing also identified a synonymous variant in *MFGE8* (p.Ser52=rs141997845_T, MAF=0.1%, β=19 ms, $P=4.9\times10^{-8}$), which was associated with marked PR interval prolongation in TOPMed (Figure 2B, Results and Table V in the Data Supplement). Only one carrier was identified in the UK Biobank, who had a first-degree atrioventricular block (PR=200 ms). In MyCode, the association was replicated (β=15 ms, $P=1.6\times10^{-3}$).

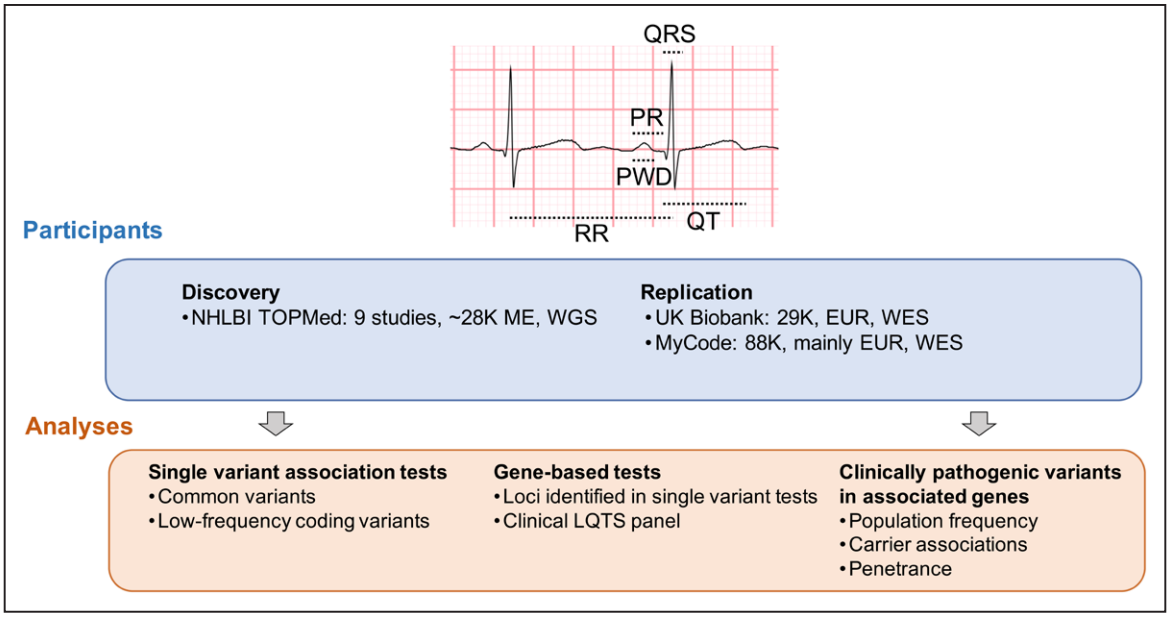

Figure 1. Flow chart of study and analyses.
Top of the figure illustrates different traits detected by the ECG. Genetic association studies were performed for 5 electrocardiographic traits in 9 studies from Trans-Omics in Precision Medicine (TOPMed) as a discovery cohort and findings from discovery analyses were replicated using UK Biobank and MyCode studies (blue). We analyzed genetic variations using both single variant association and gene-based association approaches (orange). Moreover, we calculated frequency of loss-of-function, pathogenic and likely pathogenic variants in long QT syndrome genes and performed association tests between such variants and QT interval. EUR indicates European ancestry; ME, multi-ethnic; PWD, P-wave duration; QRS, QRS duration; QTc, corrected QT interval; WES, whole-exome sequencing; and WGS, whole-genome sequencing.

Gene-based testing in TOPMed also identified 3 genes established as important for cardiac conduction, arrhythmias, and SCD (*SCN5A* [PR interval, $P=7.6\times10^{-7}$], *KCNQ1* [QTc, $P=2.3\times10^{-12}$], *KCNH2* [QTc, $P=3.2\times10^{-8}$], Figure 3, Table VI in the Data Supplement). Rare LOF variants in *SCN5A* conferred a 38 ms ($P=4.3\times10^{-32}$, N carriers=70) increase in PR interval duration across all data sets (Results, Figure VIA, and Tables VII and VIII in the Data Supplement). Furthermore, gene-based testing highlighted pleiotropy of *SCN5A*, as rare variants also associated with prolonged P-wave duration and QRS duration (Results and Figure VII in the Data Supplement). Similarly, rare LOF variants in *KCNQ1* were associated with marked prolongation of the QTc ($\beta=42$ ms, $P=3.9\times10^{-44}$, N carriers=78), as were rare LOF variants in *KCNH2* ($\beta=38$ ms, $P=8.5\times10^{-12}$, N carriers=21, Results, Figure VIB, and Tables VII and VIII in the Data Supplement). In exploratory exome-wide analyses, a number of genes reached significance (Results and Table IX in the Data Supplement). Of these genes, none reached $P<0.05$ in replication among UK Biobank participants, except for *SCN5A* (PR interval), *PAM* (PR interval), *KCNQ1* (QTc), and *KCNH2* (QTc).

In an analysis of 17 genes included on a typical clinical sequencing panel for long QT syndrome,[16] in addition to *KCNQ1* and *KCNH2*, we also identified an association between QTc and predicted-deleterious variants in *KCNE1* in TOPMed ($P=1.2\times10^{-4}$), which was replicated in the UK Biobank ($P=9.0\times10^{-5}$; Results and Table X in the Data Supplement). No *KCNE1* LOF variants were identified in TOPMed or the UK Biobank, although rare predicted-deleterious missense variants in these data sets were associated with a 16 ms ($P=3.3\times10^{-7}$) prolongation of the QTc (Results and Tables XI and XII in the Data Supplement). In contrast to the large effect sizes observed for deleterious variation in the aforementioned genes, the top variants in common variant analyses for PR interval and QTc conferred effect sizes of 4 and 3 ms, respectively (Table IV in the Data Supplement).

We observed similar findings for pathogenic or likely pathogenic variants adjudicated by clinical testing laboratories and submitted to ClinVar. Among 54 355 TOPMed and UK Biobank participants, 239 (0.44%) carried such a variant in a LQTS gene from the panel cited above. Half were located in one of the 3 most validated susceptibility genes, *KCNQ1, KCNH2,* and *SCN5A* (Table XIII in the Data Supplement).[10] Pathogenic or likely pathogenic variants in *KCNQ1* and *KCNH2* were associated with substantially prolonged QTc values across all studies (Figure VIII and Table VII in the Data Supplement). Pathogenic and likely pathogenic variants in *SCN5A* were not associated with QTc prolongation, likely owing to heterogeneity of allele effects, although were associated with substantially prolonged PR intervals (Results and Figure IXA in the Data Supplement). When aggregated together, LOF, pathogenic, or likely pathogenic variants in *KCNQ1* and *KCNH2* were associated with a 30 ms ($P=1.1\times10^{-67}$) and 27 ms ($P=1.0\times10^{-16}$) prolongation of the QTc, respectively (Figure 4).

Table. Baseline Characteristics of TOPMed Participants

ECG traits	RR interval	P-wave duration*	PR interval	QRS duration	QTc†
No. of participants	27 967	23 567	28 008	27 874	26 976
Ancestry, N (%)					
European	16 749 (59.9)	15 801 (67.0)	16 707 (59.7)	16 644 (59.7)	16 074 (59.6)
African	5034 (18.0)	4149 (17.6)	5063 (18.1)	5019 (18.0)	4887 (18.1)
Amish	1028 (3.7)	…	1024 (3.7)	1025 (3.7)	998 (3.7)
East Asian	727 (2.6)	700 (3.0)	727 (2.6)	727 (2.6)	715 (2.7)
Ad Mixed American	615 (2.2)	520 (2.2)	616 (2.2)	610 (2.2)	596 (2.2)
South Asian	56 (0.2)	…	57 (0.2)	57 (0.2)	53 (0.2)
Undetermined	3758 (13.4)	2397 (10.2)	3814 (13.6)	3792 (13.6)	3653 (13.5)
Female, N (%)	18 077 (65)	15 566 (66)	18 140 (65)	18 069 (65)	17 644 (65)
Mean age at ECG, y (SD)	60.2 (12.5)	61.1 (11.3)	60.1 (12.5)	60.1 (12.5)	59.8 (12.5)
Mean interval length, ms (SD)	937.9 (148.9)	109.5 (13.3)	164.9 (26.1)	91.0 (12.7)	423.8 (22.9)
Mean height, cm (SD)	166.0 (9.6)	166.1 (9.4)	166.0 (9.6)	166.0 (9.6)	166.0 (9.5)
Mean weight, kg (SD)	79.6 (18.8)	79.3 (18.3)	79.6 (18.8)	79.6 (18.9)	79.5 (18.9)
Myocardial infarction, N (%)	2521 (9.8)	2158 (9.2)	2503 (9.8)	2493 (9.8)	2361 (9.6)
Heart failure, N (%)	1925 (8.1)	1558 (6.7)	1914 (8.0)	1913 (8.1)	1788 (7.8)
β-Blocker, N (%)	3651 (13)	2740 (12)	3626 (13)	3638 (13)	3415 (13)
Calcium channel blocker, N (%)	3220 (12)	2632 (11)	3203 (12)	3198 (12)	3043 (12)

TOPMed indicates Trans-Omics in Precision Medicine.
*The maximum P-wave duration was obtained from the ECG leads.
†QTc is corrected QT interval using the Bazett method.

Despite the marked effect of deleterious variation on electrocardiographic intervals, incomplete penetrance was common (Table XIV in the Data Supplement). LOF, pathogenic, or likely pathogenic variants in *SCN5A*, which were carried by 0.1% of individuals, and were associated with an ≈6-fold increased odds of first-degree atrioventricular block in TOPMed and the UK Biobank (P=8.4×10^{-5}), and 12-fold increased odds in MyCode (P=2.7×10^{-12}; Tables XV in the Data Supplement). Nevertheless, about 70% of carriers had a PR interval of <200 ms, indicating absence of first-degree atrioventricular block (Results and Figure IX in the Data Supplement).

Similarly, deleterious *KCNQ1* and *KCNH2* variants were carried by 0.2% of individuals and were associated with an almost 23-fold increased odds of a QTc duration of at least 480 ms in TOPMed and UK Biobank (P=4.5×10^{-25}) and 9-fold increased odds in MyCode, an interval length that is suggestive of LQTS (Figure 5 and Table XV and Figure X in the Data Supplement).[17] Yet over 75% of individuals carrying an LOF, pathogenic, or likely pathogenic variant in *KCNQ1* or *KCNH2* had a QTc below 480 ms (95/110 in TOPMed and UK Biobank, 137/181 in MyCode).

Few individuals with prolonged intervals carried known deleterious variation. For example, among individuals with first-degree atrioventricular block, only 0.3% carried a LOF, pathogenic, or likely pathogenic variant in *SCN5A* in TOPMed and the UK Biobank, and 0.5% in MyCode. Similarly, among individuals with marked QTc prolongation (eg, ≥480ms), only 2.4% carried a LOF or known deleterious variant in *KCNQ1* or *KCNH2* in TOPMed and UK Biobank, and 1.2% in MyCode (Figure 5). Extended analyses summarizing the frequency of additional rare protein-coding variation are displayed in Table XVII in the Data Supplement. Notably, fewer than 11% of individuals with QTc≥480ms or QTc≥500 ms carried any rare *KCNQ1* or *KCNH2* protein-altering variant across TOPMed and UK Biobank.

DISCUSSION

Using a unique resource of high-depth genomic sequence data from over 130 000 individuals, we identified low-frequency and rare genetic variants underlying variability in 5 routinely collected electrocardiographic traits. Our findings indicate that pathogenic variation in arrhythmia and SCD genes are associated with marked PR (*SCN5A*) and QTc (*KCNQ1*, *KCNH2*, and *KCNE1*) prolongation in the general population. Nevertheless, over 70% of individuals with deleterious variation had normal electrocardiographic intervals, indicating that routinely measured electrocardiographic intervals may be insensitive for the detection of such carriers. Moreover, <3% of individuals with marked PR interval or QTc variation carried a known deleterious variant, and <10% with marked QTc prolongation carried any rare protein-changing variant in a predominant LQTS gene. The causes of such electrocardiographic interval prolongation remain unclear and warrant further examination. Last, our findings highlight the

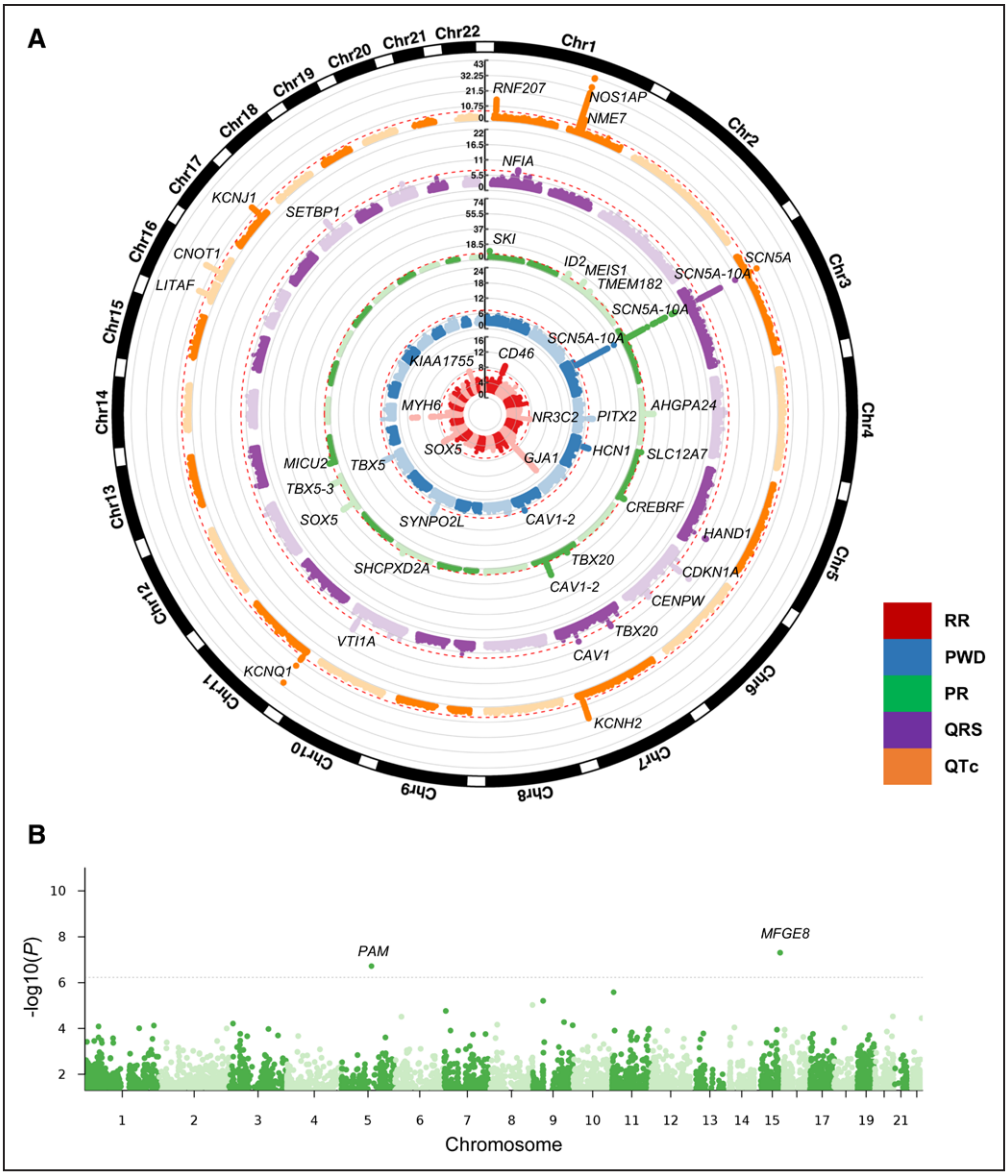

Figure 2. Manhattan plots for 5 electrocardiographic traits.
A illustrates circular Manhattan plot illustrating genome-wide association testing results between 5 electrocardiographic traits and common variants with minor allele frequency (MAF) >1%. Loci that reached a conventional genome-wide significant threshold (P=5×10^{-8}, red dotted lines) are annotated with the nearest genes. **B** shows associations between low-frequency (0.1% ≤ MAF <1%) variants and PR interval. The gray dotted line is the significant threshold (0.05/83 994 variants =6.0×10^{-7}).

value of large-scale sequencing efforts to identify novel genes, such as *PAM* and *MFGE8*, which we implicated in atrioventricular conduction.

Our study complements and extends prior literature. To date, most genome-wide association studies of electrocardiographic traits have largely been focused on common genetic variants, have studied these traits individually,[12,13,15,18–20] or have relied on sequencing in smaller samples with imputation of low-frequency variants.[14] In contrast, the recent and rapid innovation in sequencing technology has enabled the analysis of very rare and potentially deleterious coding variation in relation to cardiac traits. Such rare variants are likely to directly implicate genes in cardiac physiology and may confer large effect sizes which could be of clinical relevance.

Our findings have 3 important implications. First, rare variants in arrhythmia and SCD susceptibility genes are associated with large effects on the ECG in the population, yet routinely measured electrocardiographic intervals may not be a reliable method for identifying most carriers. Incomplete electrocardiographic penetrance was common among individuals carrying deleterious variation. Indeed, it is likely that pathogenic variation in arrhythmia and SCD genes exhibits lower penetrance than was reported previously in family-based analyses,[21–23] consistent with reports for arrhythmogenic cardiomyopathy genes.[24] Considering the frequency of

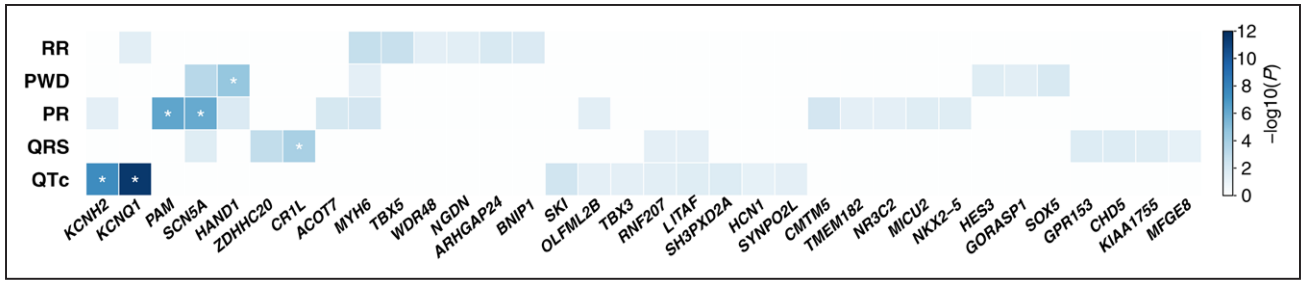

Figure 3. Association results between electrocardiographic traits and predicted-deleterious variants in genes from candidate loci.

Figure 3 illustrates associations between electrocardiographic traits (RR interval, P-wave duration [PWD], PR interval, QRS duration, and corrected QT interval [QTc]) and genes in candidate loci in Trans-Omics in Precision Medicine (TOPMed) using SMMAT. Genes with $P>0.05$ for all traits were removed from this figure. As shown in the key legend, the gradient of blue colors represents the strength of associations in this heatmap. Genes with a star (*) were significantly associated with an electrocardiographic trait ($P<1.2\times10^{-4}$); tests with $P>0.05$ have been made white. The maximum PWD was significantly associated with *HAND1* ($P=2.4\times10^{-5}$). PR interval was significantly associated with *SCN5A* ($P=7.6\times10^{-7}$) and *PAM* ($P=4.5\times10^{-7}$). QRS duration was significantly associated with *CR1L* ($P=1.2\times10^{-4}$). QTc was significantly associated with *KCNQ1* ($P=2.3\times10^{-12}$) and *KCNH2* ($P=3.2\times10^{-8}$). PR indicates PR interval; QRS, QRS duration; and RR, RR interval.

second hits that can predispose to QTc prolongation and lethal ventricular arrhythmias, the consequences of subclinical genetic predisposition to arrhythmias requires prospective evaluation, particularly given the adoption of genome-first approaches for which return of incidental findings in several arrhythmia and SCD genes is encouraged.[25] Since electrocardiographic intervals may vary over time, future analyses that leverage repeated electrocardiographic measures may provide more accurate estimates of rare variant penetrance. Moreover, analysis of additional electrocardiographic features beyond standard intervals is warranted. Importantly, whether a genome-first approach to identifying pathogenic variant carriers will have a material impact on SCD risk requires prospective evaluation.

Second, our observations suggest that quantitative traits measured in population-based studies are endophenotypes for pathogenic variation. Analyzing low-frequency and rare genetic variation in relation to commonly ascertained electrocardiographic traits is an efficient approach for identifying important genes, both established and novel, that are related to cardiac conduction and arrhythmia risk. For example, variation in *SCN5A* was associated with PR duration. *SCN5A* encodes the α-subunit of the cardiac sodium channel and comprises the major inward sodium current responsible for cardiomyocyte depolarization during phase 0. Mutations in *SCN5A* are responsible for several conditions, including atrial fibrillation, bradyarrhythmias, cardiomyopathy, Brugada syndrome, LQTS, and SCD.[11] Additionally, low-frequency and rare coding variants in *KCNQ1*, *KCNH2*, and *KCNE1* were associated with QTc. *KCNQ1* and *KCNH2* encode voltage-gated potassium channel subunits responsible for the outward rectifier currents I_{Ks} and I_{Kr},

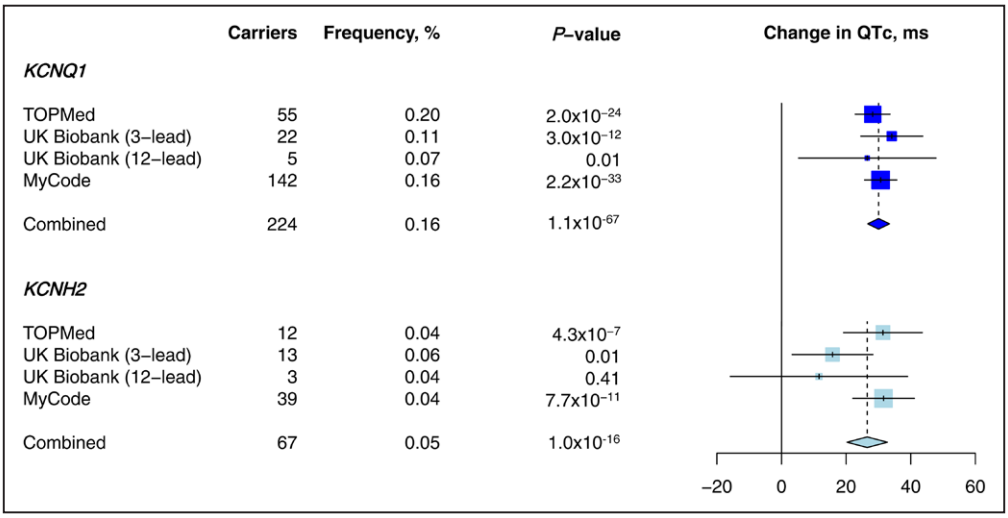

Figure 4. Forest plots for loss-of-function (LOF), pathogenic or likely pathogenic variants in *KCNQ1* and *KCNH2* and their effect on corrected QT interval (QTc).

Across Trans-Omics in Precision Medicine (TOPMed), UK Biobank and MyCode datasets, *KCNQ1* and *KCNH2* LOF and pathogenic or likely pathogenic variants significantly and markedly prolonged the QTc, with inverse-variance weighted fixed-effects meta-analyzed effect estimates of 30 ms ($P=1.1\times10^{-67}$) and 27 ms ($P=1.0\times10^{-16}$) prolongation, respectively.

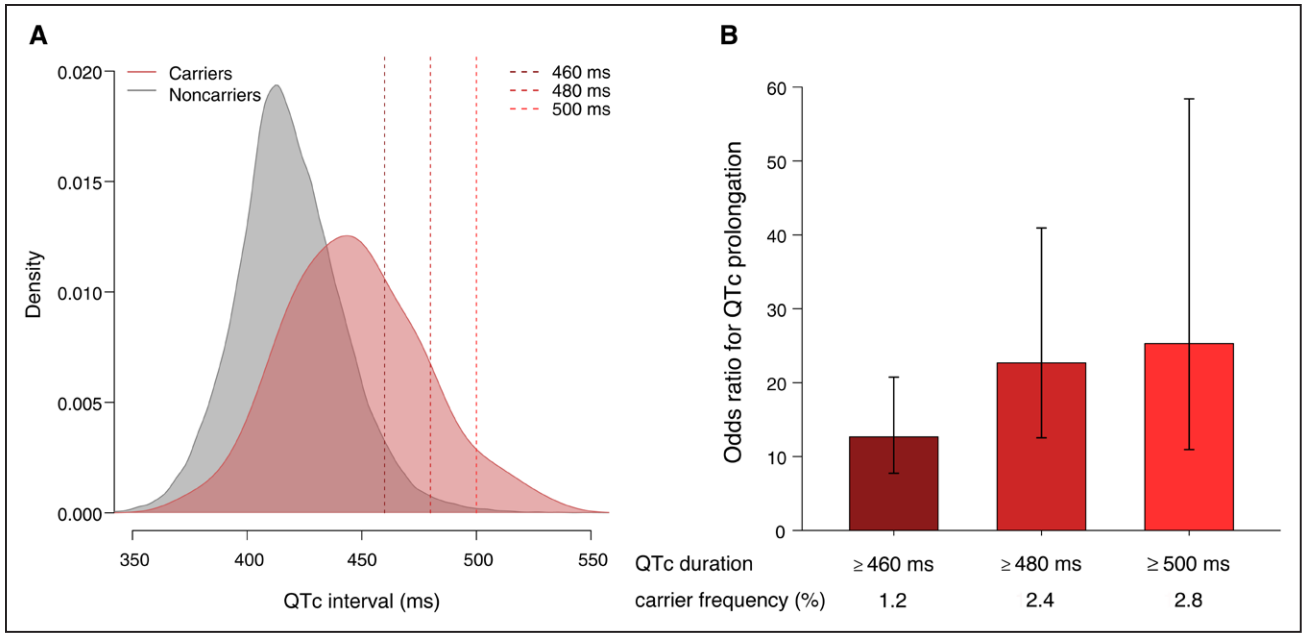

Figure 5. Effect of loss-of-function (LOF), pathogenic or likely pathogenic variants in *KCNQ1* and *KCNH2* on corrected QT interval (QTc) in the population.

A illustrates distributions for carriers (red, N=110) of a LOF or pathogenic or likely pathogenic variant in *KCNQ1*, *KCNH2*, and noncarriers (gray, N=54 245) in Trans-Omics in Precision Medicine (TOPMed) and UK Biobank. The dotted lines represent QTc cutoffs of 460, 480, and 500 ms. Of the carriers, 15 (13.6%) individuals had QTc interval ≥480 ms while 662 (1.2%) of noncarriers revealed QT prolongation. **B** illustrates the odds ratio for QTc prolongation at different cutoffs (460, 480, and 500 ms) conferred by LOF, pathogenic or likely pathogenic variants in *KCNQ1* and *KCNH2* in TOPMed and UK Biobank.

respectively, which govern cardiomyocyte repolarization during phases 2 and 3 of the action potential. Mutations in *KCNQ1* and *KCNH2* represent the most common forms of LQTS.[10] *KCNE1* encodes a β-subunit that interacts with *KCNQ1* to form the I_{Ks} current. Notably, *KCNE1* has recently been considered a controversial susceptibility gene for typical monogenic LQTS.[10,26]

Examination of sequence data in relation to ECGs can also identify novel pathways involved in cardiac physiology. *PAM* encodes peptidylglycine-alpha-amidating-monooxygenase, an enzyme expressed in atrial cardiomyocytes, where it colocalizes with atrial natriuretic peptide.[27–29] *MFGE8* encodes the milk fat globule-epidermal growth factor 8 that is involved in phagocytic signaling and has been implicated in neovascularization,[30] cardiac hypertrophy,[31] and atrial fibrosis.[32] These novel conduction genes have not been examined extensively in relation to cardiovascular disease. Future work is necessary to characterize the relations between genetic variation in these genes and disease, as well as their mechanistic roles in cardiovascular biology. Larger discovery samples anticipated in the near future are likely to identify additional arrhythmia and SCD susceptibility genes, emphasizing the importance of high-throughput functional characterization of new genes.

Third, few individuals with markedly abnormal electrocardiographic intervals had known deleterious variation in classic arrhythmia and SCD genes, indicating that the causes of such electrocardiographic variability remain unclear. We suspect that multiple factors may account for prolonged electrocardiographic intervals, including rare variants in genes not traditionally implicated in arrhythmias and SCD, polygenic susceptibility to electrocardiographic interval prolongation, and other factors, such as electrolyte abnormalities or medication exposures, that were not accounted for in our analysis. We excluded individuals on antiarrhythmic medications, individuals with paced rhythms, and pathological QRS prolongation which may confound some intervals (eg, QTc). The yield of contemporary panel-based genetic evaluations in individuals with isolated electrocardiographic interval prolongation is, therefore, likely low. The cause, prognosis, and optimal management of these genotype-negative individuals in the community warrants evaluation considering the adverse prognosis traditionally associated with both prolonged PR interval and QTc.[3,8,33–36]

Our results should be evaluated in the context of the study design. The sample consisted mainly of middle-aged individuals of European ancestry, limiting generalizability of results beyond the ancestral groups and age strata represented. We used single time point electrocardiographic analyses and intervals may vary over time, although for the studied traits 10 second ECGs have been determined to be reliable.[37,38] We did not account for all medications that may affect cardiac conduction, which requires future investigation. Finally, we cannot exclude a survival bias since included participants were adults at the time of enrollment.

In conclusion, we demonstrate the value of large-scale high-depth sequence analysis for interrogating the genetic basis of the ECG. As biorepositories grow in the near future, similar approaches will undoubtedly uncover additional rare variants with other high-impact on cardiovascular diseases.

ARTICLE INFORMATION

Received August 18, 2020; accepted July 11, 2021.

Affiliations

Program in Medical and Population Genetics and Cardiovascular Disease Initiative, Broad Institute of MIT and Harvard, Cambridge, MA (S.H.C., S.J.J., A.W.H., J.L.H., V.N.M., L.-C.W., M.D.C., C.J.-Y.L., H.L.R., C.R., P.T.E., S.A.L.). Department of Translational Data Science and Informatics (C.M.H., B.K.F.), Heart Institute (C.M.H., B.K.F.), Phenomic Analytics and Clinical Data Core (B.L.), Department of Molecular and Functional Genomics (T.M.), and Department of Radiology (B.K.F.), Geisinger, Danville, PA. Cardiovascular Research Center (A.W.H., V.N.M., L.-C.W., P.T.E., S.A.L.), Center for Genomic Medicine (C.A.-T., H.L.R.), and Cardiac Arrhythmia Service (P.T.E., S.A.L.), Massachusetts General Hospital. Division of Sleep Medicine, Department of Medicine (B.E.C.) and Harvard Medical School (J.L.H., C.A.-T., H.L.R.), Boston, MA. Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA (M.P., C.K.). The Institute for Translational Genomics and Population Sciences, Department of Pediatrics, The Lundquist Insti for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA (X.G., H.J.L., J.I.R.). Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA (A.A.). Epidemiological Cardiology Research Center, Wake Forest School of Medicine, Winston Salem, NC (E.Z.S.). NHLBI and Boston University's Framingham Heart Study (J.K., E.J.B., R.S.V). Sections of Cardiovascular Medicine and Preventive Medicine, Boston Medical Center (J.K., R.S.V), Section of Computational Biomedicine, Department of Medicine (H.L.), and Department of Medicine (E.J.B., R.S.V), Boston University School of Medicine, MA. The Charles Bronfman Institute for Personalized Medicine (A.M., G.N., R.J.F.L.), Division of Nephrology, Department of Medicine (G.N.), and The Mindich Child Health and Development Institute (R.J.F.L.), Icahn School of Medicine, Mount Sinai, New York, NY. Cardiovascular Health Research Unit, Department of Medicine (J.A.B., K.L.W., N.S., B.M.P., S.R.H.), Division of Cardiology, Department of Epidemiology (N.S.), Department of Epidemiology (B.M.P., S.R.H.), and Department of Health Services (B.M.P.), University of Washington, Seattle. Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology (B.E.C.) and Division of Sleep and Circadian Disorders (J.L.), Harvard Medical School, Brigham and Women's Hospital, Boston. Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, Cambridge, MA (C.A.-T.). Department of Biostatistics, University of Michigan, Ann Arbor (T.B.). Regeneron Genetics Center, Tarrytown, NY. Departments of Medicine, Brigham and Women's Hospital, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA (S.R.). University of Maryland School of Medicine (B.D.M.). Geriatrics Research and Education Clinical Center, Baltimore Veterans Administration Medical Center, MD (B.D.M.). Kaiser Permanente Washington Health Research Institute, Seattle (B.M.P.). Department of Epidemiology (E.J.B.) and Department of Biostatistics (K.L.L.), Boston University School of Public Health, MA. Departments of Medicine, Pediatrics, and Population Health Science, University of Mississippi Medical Center, Jackson (A.C.). Human Genetics Center, University of Texas Health Science Center, Houston (E.B.). McKusick-Nathans Institute, Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD (D.E.A.). Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville (S.S.R.). Department of Epidemiology, Gillings School of Global Public Health (E.A.W.) and Department of Medicine (E.A.W.), School of Medicine, University of North Carolina, Chapel Hill. Division of Cardiovascular Medicine, Stanford University, CA (M.P.). Dr Sotoodehnia is supported by NIH grant R01HL141989, by AHA grant 19SFRN34830063, and by the Laughlin Family.

Acknowledgments

We gratefully acknowledge the studies and participants who provided biological samples and data for TOPMed and the DiscovEHR.

Sources of Funding

The whole-genome sequencing for the Trans-Omics in Precision Medicine (TOPMed) program was supported by the National Heart, Lung, and Blood Institute (NHLBI). Whole-genome sequencing for NHLBI TOPMed: the Atherosclerosis Risk in Communities (phs001211.v1.p1) was performed at the Broad Institute of MIT and Harvard (3R01HL092577-06S1) and Baylor Human Genome Sequencing Center (3U54HG003273-12S2 and HHSN268201500015C). Whole-genome sequencing for NHLBI TOPMed: Genetics of Cardiometabolic Health in the Amish (phs000956.v1.p1) was performed at the Broad Institute of MIT and Harvard (3R01HL121007-01S1). Whole-genome sequencing for NHLBI TOPMed: Mount Sinai BioMe Biobank (phs001644.v1.p1) was performed at the Baylor Human Genome Sequencing Center (HHSN268201600033I) and McDonnell genome institute (HHSN268201600037I). Whole-genome sequencing for NHLBI TOPMed: Cleveland Family Study—Whole-genome sequencing collaboration (phs000954.v1.p1) was performed at the University of Washington northwest genomics center (3R01HL098433-05S1 and HHSN268201600032I). Whole-genome sequencing for NHLBI TOPMed: Cardiovascular Health Study (phs001368.v1.p1) was performed at the Baylor Human Genome Sequencing Center (3U54HG003273-12S2, HHSN268201500015C, and HHSN268201600033I). Whole-genome sequencing for NHLBI TOPMed: Framingham Heart Study (phs000974.v1.p1) was performed at the Broad Institute of MIT and Harvard (3R01HL092577-06S1 and 3U54HG003067-12S2). Whole-genome sequencing for NHLBI TOPMed: The Jackson Heart Study (phs000964.v1.p1) was performed at the University of Washington Northwest Genomics Center (HHSN268201100037C). Whole-genome sequencing for NHLBI TOPMed: Multi-Ethnic Study of Atherosclerosis (phs001416.v1.p1) was performed at the Broad Institute of MIT and Harvard (3U54HG003067-13S1). Whole-genome sequencing for NHLBI TOPMed: Womens Health Initiative (phs001237.v1.p1) was performed at the Broad Institute of MIT and Harvard (HHSN268201500014C). Centralized read mapping and genotype calling, along with variant quality metrics and filtering were provided by the TOPMed Informatics Research Center (3R01HL-117626-02S1; contract HHSN268201800002I). Phenotype harmonization, data management, sample-identity QC, and general study coordination were provided by the TOPMed Data Coordinating Center (3R01HL-120393-02S1; contract HHSN268201800001I). The TOPMed component of the Amish Research Program was supported by the National Institutes of Health (NIH) grants R01 HL121007, U01 HL072515, and R01AG18728. The Mount Sinai BioMe Biobank has been supported by The Andrea and Charles Bronfman Philanthropies and, in part, by Federal funds from the NHLBI and NHGRI (U01HG00638001; U01HG007417; and X01HL134588). The ARIC study (Atherosclerosis Risk in Communities) has been funded in whole or in part with Federal funds from the NHLBI, NIH, Department of Health and Human Services (contract numbers HHSN268201700001I, HHSN268201700002I, HHSN268201700003I, HHSN268201700004I, and HHSN268201700005I). The CHS (Cardiovascular Health Study) was supported by contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, 75N92021D00006, and grants U01HL080295 and U01HL130114 from the NHLBI, with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The CFS (Cleveland Family Study) has been supported, in part, by NIH grants (R01-HL046380, KL2-RR024990, R35-HL135818, and R01-HL113338). The FHS (Framingham Heart Study) acknowledges the support of contracts NO1-HC-25195 and HHSN268201500001 from the NHLBI and grant supplement R01 HL092577-06S1, and 18SFRN34110082 from American Heart Association for this research. We also acknowledge the dedication of the FHS study participants without whom this research would not be possible. Dr Vasan is supported, in part, by the Evans Medical Foundation and the Jay and Louis Coffman Endowment from the Department of Medicine, Boston University School of Medicine. Dr Kornej has received funding from the Marie Sklodowska-Curie Actions under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 838259). Dr Lunetta acknowledges support under contract R01 HL092577-10. Dr Benjamin is supported under contracts R01HL128914, 2R01 HL092577 from the NHLBI and 18SFRN34110082 from the American Heart Association. The JHS (Jackson Heart Study) is supported and conducted in collaboration with Jackson State University (HHSN268201800013I), Tougaloo College (HHSN268201800014I), the Mississippi State Department of Health (HHSN268201800015I) and the University of Mississippi Medical Center (HHSN268201800010I, HHSN268201800011I, and HHSN268201800012I) contracts from the NHLBI and the National Institute on Minority Health and Health Disparities (NIMHD). MESA (Multi-Ethnic Study of Atherosclerosis) and the MESA SHARe project are conducted and supported by the NHLBI in collaboration with MESA investigators. Support for MESA is provided by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165,

N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420. MESA family is conducted and supported by the NHLBI in collaboration with MESA investigators. Support is provided by grants and contracts R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071258, and R01HL071259 and by the National Center for Research Resources, Grant UL1RR033176. The provision of genotyping data was supported, in part, by the National Center for Advancing Translational Sciences, CTSI grant UL1TR001881, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The Women's Health Initiative program is funded by the NHLBI, NIH, US Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C. We would further like to thank Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller from the NHLBI program office, Garnet Anderson, Ross Prentice, and Andrea LaCroix from the Clinical Coordinating Center (Fred Hutchinson Cancer Research Center, Seattle, WA), Investigators JoAnn E. Manson (Brigham and Women's Hospital, Harvard Medical School, Boston, MA), Barbara V. Howard (MedStar Health Research Institute/Howard University, Washington, DC), Marcia L. Stefanick (Stanford Prevention Research Center, Stanford, CA), Rebecca Jackson (The Ohio State University, Columbus, OH), Cynthia A. Thomson (University of Arizona, Tucson/Phoenix, AZ), Jean Wactawski-Wende (University at Buffalo, Buffalo, NY), Marian Limacher (University of Florida, Gainesville/Jacksonville, FL), Jennifer Robinson (University of Iowa, Iowa City/Davenport, IA), Lewis Kuller (University of Pittsburgh, Pittsburgh, PA), Sally Shumaker (Wake Forest University School of Medicine, Winston-Salem, NC), Robert Brunner (University of Nevada, Reno, NV), and Mark Espeland from the Women's Health Initiative Memory Study (Wake Forest University School of Medicine, Winston-Salem, NC). The DiscovEHR (MyCode) study was supported by the NHLBI through grant number R01HL141901. Dr Lubitz is supported by NIH grant 1R01HL139731 and American Heart Association 18SFRN34250007. Dr Sotoodehnia is supported by NIH grant R01HL141989, by AHA grant 19SFRN34830063, and by the Laughlin Family.

Disclosures

Dr Lubitz receives sponsored research support from Bristol Myers Squibb/Pfizer, Bayer AG, Boehringer Ingelheim, and Fitbit and has consulted for Bristol Myers Squibb/Pfizer and Bayer AG and participates in a research collaboration with IBM. Dr Ellinor is the principal investigator on a grant at the Broad Institute from Bayer HealthCare.

Supplemental Materials

Supplemental Methods
Extended Supplemental Methods and Results
Description of TOPMed participating studies
Banner Authors
Supplemental Acknowledgments
Supplemental Figures I–XIII
Supplemental Tables I–XVII
References [39–64]

REFERENCES

1. Bogle BM, Ning H, Mehrotra S, Goldberger JJ, Lloyd-Jones DM. Lifetime risk for sudden cardiac death in the community. *J Am Heart Assoc.* 2016;5:e002398. doi: 10.1161/JAHA.115.002398
2. Weng LC, Preis SR, Hulme OL, Larson MG, Choi SH, Wang B, Trinquart L, McManus DD, Staerk L, Lin H, et al. Genetic predisposition, clinical risk factor Burden, and lifetime risk of atrial fibrillation. *Circulation.* 2018;137:1027–1038. doi: 10.1161/CIRCULATIONAHA.117.031431
3. Cheng S, Keyes MJ, Larson MG, McCabe EL, Newton-Cheh C, Levy D, Benjamin EJ, Vasan RS, Wang TJ. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. *JAMA.* 2009;301:2571–2577. doi: 10.1001/jama.2009.888
4. Kurl S, Mäkikallio TH, Rautaharju P, Kiviniemi V, Laukkanen JA. Duration of QRS complex in resting electrocardiogram is a predictor of sudden cardiac death in men. *Circulation.* 2012;125:2588–2594. doi: 10.1161/CIRCULATIONAHA.111.025577
5. Nielsen JB, Kühl JT, Pietersen A, Graff C, Lind B, Struijk JJ, Olesen MS, Sinner MF, Bachmann TN, Haunsø S, et al. P-wave duration and the risk of atrial fibrillation: results from the Copenhagen ECG Study. *Heart Rhythm.* 2015;12:1887–1895. doi: 10.1016/j.hrthm.2015.04.026
6. Finocchiaro G, Sheikh N, Biagini E, Papadakis M, Maurizi N, Sinagra G, Pelliccia A, Rapezzi C, Sharma S, Olivotto I. The electrocardiogram in the diagnosis and management of patients with hypertrophic cardiomyopathy. *Heart Rhythm.* 2020;17:142–151. doi: 10.1016/j.hrthm.2019.07.019
7. Marcus FI. Electrocardiographic features of inherited diseases that predispose to the development of cardiac arrhythmias, long QT syndrome, arrhythmogenic right ventricular cardiomyopathy/dysplasia, and Brugada syndrome. *J Electrocardiol.* 2000;33(Suppl):1–10. doi: 10.1054/jelc.2000.20360
8. Zhang Y, Post WS, Blasco-Colmenares E, Dalal D, Tomaselli GF, Guallar E. Electrocardiographic QT interval and mortality: a meta-analysis. *Epidemiology.* 2011;22:660–670. doi: 10.1097/EDE.0b013e318225768b
9. Nolte IM, Jansweijer JA, Riese H, Asselbergs FW, van der Harst P, Spector TD, Pinto YM, Snieder H, Jamshidi Y. A comparison of heritability estimates by classical twin modeling and based on genome-wide genetic relatedness for cardiac conduction traits. *Twin Res Hum Genet.* 2017;20:489–498. doi: 10.1017/thg.2017.55
10. Adler A, Novelli V, Amin AS, Abiusi E, Care M, Nannenberg EA, Feilotter H, Amenta S, Mazza D, Bikker H, et al. An international, multicentered, evidence-based reappraisal of genes reported to cause congenital long QT syndrome. *Circulation.* 2020;141:418–428. doi: 10.1161/CIRCULATIONAHA.119.043132
11. Wilde AAM, Amin AS. Clinical spectrum of SCN5A mutations: long QT syndrome, brugada syndrome, and cardiomyopathy. *JACC Clin Electrophysiol.* 2018;4:569–579. doi: 10.1016/j.jacep.2018.03.006
12. van Setten J, Brody JA, Jamshidi Y, Swenson BR, Butler AM, Campbell H, Del Greco FM, Evans DS, Gibson Q, Gudbjartsson DF, et al. PR interval genome-wide association meta-analysis identifies 50 loci associated with atrial and atrioventricular electrical activity. *Nat Commun.* 2018;9:2904. doi: 10.1038/s41467-018-04766-9
13. Arking DE, Pulit SL, Crotti L, van der Harst P, Munroe PB, Koopmann TT, Sotoodehnia N, Rossin EJ, Morley M, Wang X, et al; CARe Consortium; COGENT Consortium; DCCT/EDIC; eMERGE Consortium; HRGEN Consortium. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. *Nat Genet.* 2014;46:826–836. doi: 10.1038/ng.3014
14. Norland K, Sveinbjornsson G, Thorolfsdottir RB, Davidsson OB, Tragante V, Rajamani S, Helgadottir A, Gretarsdottir S, van Setten J, Asselbergs FW, et al. Sequence variants with large effects on cardiac electrophysiology and disease. *Nat Commun.* 2019;10:4803. doi: 10.1038/s41467-019-12682-9
15. den Hoed M, Eijgelsheim M, Esko T, Brundel BJ, Peal DS, Evans DM, Nolte IM, Segrè AV, Holm H, Handsaker RE, et al; Global BPgen Consortium; CARDIoGRAM Consortium; PR GWAS Consortium; QRS GWAS Consortium; QT-IGC Consortium; CHARGE-AF Consortium. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nat Genet.* 2013;45:621–631. doi: 10.1038/ng.2610
16. Invitae Long QT Syndrome Panel. 2020. Accessed February 27, 2020. https://www.invitae.com/en/physician/tests/02211/
17. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, Elliott PM, Fitzsimons D, Hatala R, Hindricks G, et al; ESC Scientific Document Group. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). *Eur Heart J.* 2015;36:2793–2867. doi: 10.1093/eurheartj/ehv316
18. van der Harst P, van Setten J, Verweij N, Vogler G, Franke L, Maurano MT, Wang X, Mateo Leach I, Eijgelsheim M, Sotoodehnia N, et al. 52 genetic loci influencing myocardial mass. *J Am Coll Cardiol.* 2016;68:1435–1448. doi: 10.1016/j.jacc.2016.07.729
19. Nolte IM, Munoz ML, Tragante V, Amare AT, Jansen R, Vaez A, von der Heyde B, Avery CL, Bis JC, Dierckx B, et al. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nat Commun.* 2017;8:15805. doi: 10.1038/ncomms15805
20. Ntalla I, Weng LC, Cartwright JH, Hall AW, Sveinbjornsson G, Tucker NR, Choi SH, Chaffin MD, Roselli C, Barnes MR, et al. Multi-ancestry GWAS of the electrocardiographic PR interval identifies 202 loci underlying cardiac conduction. *Nat Commun.* 2020;11:2542. doi: 10.1038/s41467-020-15706-x
21. Vincent GM, Timothy KW, Leppert M, Keating M. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. *N Engl J Med.* 1992;327:846–852. doi: 10.1056/NEJM199209173271204
22. Viadero MT, Rubín E, Amigo T, González-Lamuño D. Three generations of hereditary long-QT syndrome with complete penetrance caused by

the p.G316E KCNQ1 mutation. *Pediatr Cardiol.* 2011;32:102–104. doi: 10.1007/s00246-010-9821-7
23. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. *Circulation.* 1999;99:529–533. doi: 10.1161/01.cir.99.4.529
24. Haggerty CM, James CA, Calkins H, Tichnell C, Leader JB, Hartzel DN, Nevius CD, Pendergrass SA, Person TN, Schwartz M, et al. Electronic health record phenotype in subjects with genetic variants associated with arrhythmogenic right ventricular cardiomyopathy: a study of 30,716 subjects with exome sequencing. *Genet Med.* 2017;19:1245–1252. doi: 10.1038/gim.2017.40
25. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* 2017;19:249–255. doi: 10.1038/gim.2016.190
26. Roberts JD, Asaki SY, Mazzanti A, Bos JM, Tuleta I, Muir AR, Crotti L, Krahn AD, Kutyifa V, Shoemaker MB, et al. An international multicenter evaluation of type 5 long QT syndrome: a low penetrant primary arrhythmic condition. *Circulation.* 2020;141:429–439. doi: 10.1161/CIRCULATIONAHA.119.043114
27. O'Donnell PJ, Driscoll WJ, Bäck N, Muth E, Mueller GP. Peptidylglycine-alpha-amidating monooxygenase and pro-atrial natriuretic peptide constitute the major membrane-associated proteins of rat atrial secretory granules. *J Mol Cell Cardiol.* 2003;35:915–922. doi: 10.1016/s0022-2828(03)00171-8
28. Ouafik L, May V, Keutmann HT, Eipper BA. Developmental regulation of peptidylglycine alpha-amidating monooxygenase (PAM) in rat heart atrium and ventricle. Tissue-specific changes in distribution of PAM activity, mRNA levels, and protein forms. *J Biol Chem.* 1989;264:5839–5845.
29. Doll S, Dreßen M, Geyer PE, Itzhak DN, Braun C, Doppler SA, Meier F, Deutsch MA, Lahm H, Lange R, et al. Region and cell-type resolved quantitative proteomic map of the human heart. *Nat Commun.* 2017;8:1469. doi: 10.1038/s41467-017-01747-2
30. Silvestre JS, Théry C, Hamard G, Boddaert J, Aguilar B, Delcayre A, Houbron C, Tamarat R, Blanc-Brude O, Heeneman S, et al. Lactadherin promotes VEGF-dependent neovascularization. *Nat Med.* 2005;11:499–506. doi: 10.1038/nm1233
31. Deng KQ, Li J, She ZG, Gong J, Cheng WL, Gong FH, Zhu XY, Zhang Y, Wang Z, Li H. Restoration of circulating MFGE8 (Milk Fat Globule-EGF Factor 8) attenuates cardiac hypertrophy through inhibition of Akt pathway. *Hypertension.* 2017;70:770–779. doi: 10.1161/HYPERTENSIONAHA.117.09465
32. Ge Z, Chen Y, Wang B, Zhang X, Yan Y, Zhou L, Zhang Y, Xie Y. MFGE8 attenuates Ang-II-induced atrial fibrosis and vulnerability to atrial fibrillation through inhibition of TGF-β1/Smad2/3 pathway. *J Mol Cell Cardiol.* 2020;139:164–175. doi: 10.1016/j.yjmcc.2020.01.001
33. Straus SM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MC, Stricker BH, et al. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol.* 2006;47:362–367. doi: 10.1016/j.jacc.2005.08.067
34. Robbins J, Nelson JC, Rautaharju PM, Gottdiener JS. The association between the length of the QT interval and mortality in the Cardiovascular Health Study. *Am J Med.* 2003;115:689–694. doi: 10.1016/j.amjmed.2003.07.014
35. Panikkath R, Reinier K, Uy-Evanado A, Teodorescu C, Gunson K, Jui J, Chugh SS. Electrocardiographic predictors of sudden cardiac death in patients with left ventricular hypertrophy. *Ann Noninvasive Electrocardiol.* 2013;18:225–229. doi: 10.1111/anec.12003
36. Zhang Y, Post WS, Dalal D, Blasco-Colmenares E, Tomaselli GF, Guallar E. QT-interval duration and mortality rate: results from the Third National Health and Nutrition Examination Survey. *Arch Intern Med.* 2011;171:1727–1733. doi: 10.1001/archinternmed.2011.433
37. Snyder ML, Soliman EZ, Whitsel EA, Gellert KS, Heiss G. Short-term repeatability of electrocardiographic P wave indices and PR interval. *J Electrocardiol.* 2014;47:257–263. doi: 10.1016/j.jelectrocard.2013.11.007
38. Gellert KS, Rautaharju P, Snyder ML, Whitsel EA, Matsushita K, Heiss G, Soliman EZ. Short-term repeatability of electrocardiographic Tpeak-Tend and QT intervals. *J Electrocardiol.* 2014;47:356–361. doi: 10.1016/j.jelectrocard.2014.03.002
39. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature.* 2018;562:203–209. doi: 10.1038/s41586-018-0579-z
40. Van Hout VC, Tachmazidou I, Backman JD, Hoffman JX, Ye B, Pandey AK, Gonzaga-Jauregui C, Khalid SL, Liu D, Banerjee N, Li AH, et al. Whole exome sequencing and characterization of coding variation in 49,960 individuals in the UK Biobank. *bioRxiv.* Preprint posted online March 9, 2019. doi: 10.1101/572347
41. Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. Robust relationship inference in genome-wide association studies. *Bioinformatics.* 2010;26:2867–2873. doi: 10.1093/bioinformatics/btq559
42. Gogarten SM, Sofer T, Chen H, Yu C, Brody JA, Thornton TA, Rice KM, Conomos MP. Genetic association testing using the GENESIS R/Bioconductor package. *Bioinformatics.* 2019;35:5346–5348. doi: 10.1093/bioinformatics/btz567
43. Abraham G, Qiu Y, Inouye M. FlashPCA2: principal component analysis of Biobank-scale genotype datasets. *Bioinformatics.* 2017;33:2776–2778. doi: 10.1093/bioinformatics/btx299
44. Zhou W, Nielsen JB, Fritsche LG, Dey R, Gabrielsen ME, Wolford BN, LeFaive J, VandeHaar P, Gagliano SA, Gifford A, et al. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nat Genet.* 2018;50:1335–1341. doi: 10.1038/s41588-018-0184-y
45. Choi SH, Weng LC, Roselli C, Lin H, Haggerty CM, Shoemaker MB, Barnard J, Arking DE, Chasman DI, Albert CM, et al; DiscovEHR study and the NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium. Association between titin loss-of-function variants and early-onset atrial fibrillation. *JAMA.* 2018;320:2354–2364. doi: 10.1001/jama.2018.18179
46. Chen H, Huffman JE, Brody JA, Wang C, Lee S, Li Z, Gogarten SM, Sofer T, Bielak LF, Bis JC, et al; NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium; TOPMed Hematology and Hemostasis Working Group. Efficient variant set mixed model association tests for continuous and binary traits in large-scale whole-genome sequencing studies. *Am J Hum Genet.* 2019;104:260–274. doi: 10.1016/j.ajhg.2018.12.012
47. Chen H, Wang C, Conomos MP, Stilp AM, Li Z, Sofer T, Szpiro AA, Chen W, Brehm JM, Celedón JC, et al. Control for population structure and relatedness for binary traits in genetic association studies via logistic mixed models. *Am J Hum Genet.* 2016;98:653–666. doi: 10.1016/j.ajhg.2016.02.012
48. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, et al; Genome Aggregation Database Consortium. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature.* 2020;581:434–443. doi: 10.1038/s41586-020-2308-7
49. Liu X, Wu C, Li C, Boerwinkle E. dbNSFP v3.0: a one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Hum Mutat.* 2016;37:235–241. doi: 10.1002/humu.22932
50. Heinze G. A comparative investigation of methods for logistic regression with separated or nearly separated data. *Stat Med.* 2006;25:4216–4226. doi: 10.1002/sim.2687
51. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience.* 2015;4:7. doi: 10.1186/s13742-015-0047-8
52. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 2009;19:1655–1664. doi: 10.1101/gr.094052.109
53. Aragam KG, Chaffin M, Levinson RT, McDermott G, Choi SH, Shoemaker MB, Haas ME, Weng LC, Lindsay ME, Smith JG, et al. Phenotypic refinement of heart failure in a National Biobank Facilitates Genetic Discovery. *Circulation.* 2019;139:489–501. doi: 10.1161/CIRCULATIONAHA.118.035774
54. Regier AA, Farjoun Y, Larson DE, Krasheninina O, Kang HM, Howrigan DP, Chen BJ, Kher M, Banks E, Ames DC, et al. Functional equivalence of genome sequencing analysis pipelines enables harmonized variant calling across human genetics projects. *Nat Commun.* 2018;9:4038. doi: 10.1038/s41467-018-06159-4
55. Staples J, Maxwell EK, Gosalia N, Gonzaga-Jauregui C, Snyder C, Hawes A, Penn J, Ulloa R, Bai X, Lopez AE, et al. Profiling and leveraging relatedness in a precision medicine cohort of 92,455 exomes. *Am J Hum Genet.* 2018;102:874–889. doi: 10.1016/j.ajhg.2018.03.012
56. Thomsen SK, Raimondo A, Hastoy B, Sengupta S, Dai XQ, Bautista A, Censin J, Payne AJ, Umapathysivam MM, Spigelman AF, et al. Type 2 diabetes risk alleles in PAM impact insulin release from human pancreatic β-cells. *Nat Genet.* 2018;50:1122–1131. doi: 10.1038/s41588-018-0173-1
57. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet.* 2015;47:1121–1130. doi: 10.1038/ng.3396

58. Chen B, Huang S, Su Y, Wu YJ, Hanna A, Brickshawana A, Graff J, Frangogiannis NG. Macrophage Smad3 protects the infarcted heart, stimulating phagocytosis and regulating inflammation. *Circ Res.* 2019;125:55–70. doi: 10.1161/CIRCRESAHA.119.315069
59. Firulli BA, George RM, Harkin J, Toolan KP, Gao H, Liu Y, Zhang W, Field LJ, Liu Y, Shou W, et al. HAND1 loss-of-function within the embryonic myocardium reveals survivable congenital cardiac defects and adult heart failure. *Cardiovasc Res.* 2020;116:605–618. doi: 10.1093/cvr/cvz182
60. Holm H, Gudbjartsson DF, Sulem P, Masson G, Helgadottir HT, Zanon C, Magnusson OT, Helgason A, Saemundsdottir J, Gylfason A, et al. A rare variant in MYH6 is associated with high risk of sick sinus syndrome. *Nat Genet.* 2011;43:316–320. doi: 10.1038/ng.781
61. Lin H, van Setten J, Smith AV, Bihlmeyer NA, Warren HR, Brody JA, Radmanesh F, Hall L, Grarup N, Müller-Nurasyid M, et al. Common and rare coding genetic variation underlying the electrocardiographic PR interval. *Circ Genom Precis Med.* 2018;11:e002037. doi: 10.1161/CIRCGEN.117.002037
62. Carniel E, Taylor MR, Sinagra G, Di Lenarda A, Ku L, Fain PR, Boucek MM, Cavanaugh J, Miocic S, Slavov D, et al. Alpha-myosin heavy chain: a sarcomeric gene associated with dilated and hypertrophic phenotypes of cardiomyopathy. *Circulation.* 2005;112:54–59. doi: 10.1161/CIRCULATIONAHA.104.507699
63. Granados-Riveron JT, Ghosh TK, Pope M, Bu'Lock F, Thornborough C, Eason J, Kirk EP, Fatkin D, Feneley MP, Harvey RP, et al. Alpha-cardiac myosin heavy chain (MYH6) mutations affecting myofibril formation are associated with congenital heart defects. *Hum Mol Genet.* 2010;19:4007–4016. doi: 10.1093/hmg/ddq315
64. Brugada J, Campuzano O, Arbelo E, Sarquella-Brugada G, Brugada R. Present status of Brugada syndrome: JACC state-of-the-art review. *J Am Coll Cardiol.* 2018;72:1046–1059. doi: 10.1016/j.jacc.2018.06.037