52 Genetic Loci Influencing Myocardial Mass

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ABSTRACT

BACKGROUND Myocardial mass is a key determinant of cardiac muscle function and hypertrophy. Myocardial depolarization leading to cardiac muscle contraction is reflected by the amplitude and duration of the QRS complex on the electrocardiogram (ECG). Abnormal QRS amplitude or duration reflect changes in myocardial mass and conduction, and are associated with increased risk of heart failure and death.

OBJECTIVES This meta-analysis sought to gain insights into the genetic determinants of myocardial mass.

METHODS We carried out a genome-wide association meta-analysis of 4 QRS traits in up to 73,518 individuals of European ancestry, followed by extensive biological and functional assessment.

RESULTS We identified 52 genomic loci, of which 32 are novel, that are reliably associated with 1 or more QRS phenotypes at $p < 1 \times 10^{-8}$. These loci are enriched in regions of open chromatin, histone modifications, and transcription factor binding, suggesting that they represent regions of the genome that are actively transcribed in the human heart. Pathway analyses provided evidence that these loci play a role in cardiac hypertrophy. We further highlighted 67 candidate genes at the identified loci that are preferentially expressed in cardiac tissue and associated with cardiac abnormalities in *Drosophila melanogaster* and *Mus musculus*. We validated the regulatory function of a novel variant in the *SCN5A/SCN10A* locus in vitro and in vivo.

CONCLUSIONS Taken together, our findings provide new insights into genes and biological pathways controlling myocardial mass and may help identify novel therapeutic targets. (J Am Coll Cardiol 2016;68:1435–48)
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The heart's role is to provide adequate circulation of blood to meet the body's requirements of oxygen and nutrients. The QRS complex on the electrocardiogram (ECG) represents the most widely used measurement of cardiac depolarization, which causes the ventricular muscle to contract, resulting in pulsatile blood flow. The amplitude and duration of the QRS complex reflects conduction through the left ventricle and is well correlated with left ventricular mass as measured by echocardiography [1,2]. ECG measurements of the QRS complex are important in clinical and pre-clinical cardiovascular

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ABBREVIATIONS AND ACRONYMS

DHS = deoxyribonuclease hypersensitivity sites

ECG = electrocardiogram

eQTL = expression quantitative trait locus

GWAS = genome-wide association study

LD = linkage disequilibrium

RNAi = ribonucleic acid interference

SNP = single nucleotide polymorphism

TF = transcription factor

diseases, such as cardiac hypertrophy, heart failure, and various cardiomyopathies; in addition, they can predict cardiovascular mortality (3-6).

SEE PAGE 1449

Identification of specific genes influencing the QRS complex may thus enhance our understanding of the human heart and ultimately lead to the prevention of cardiovascular disease and death. To further our understanding of the genetic factors influencing the QRS complex, we carried out a large-scale genome-wide association study (GWAS) and replication study of 4 related and clinically used QRS traits: the Sokolow-Lyon, Cornell, and 12-lead-voltage duration products (12-leadsum), and QRS duration. We identified 52 loci that were subsequently interrogated using bioinformatics and experimental approaches to gain more insights into the biological mechanisms regulating cardiac mass and QRS parameters.

METHODS

Additional details about the methods of our study can be found in the Online Appendix.

Our study design is summarized in Online Figure 1. Briefly, we combined summary statistics from 24 studies for up to 2,766,983 autosomal single nucleotide polymorphisms (SNPs) using an inverse-variance fixed-effects meta-analysis for each QRS trait. We performed replication testing for loci showing suggestive association ($1 \times 10^{-8} < p < 5 \times 10^{-7}$) (Online Tables 1 and 2). The threshold for genome-wide significance was set at $p < 1 \times 10^{-8}$.

We performed an intersection between SNPs and regions of deoxyribonuclease hypersensitivity sites (DHS), covalently modified histones, and genomic features (ChromHMM) of cardiac tissues mapped by the National Institutes of Health Roadmap Epigenomics Program, as well as various cardiac transcription factor binding sites (GATA4, MEF2, SRF, TBX5, TBX3, GATA4, and Nkx2-5) measured by Chip-seq.

Single cell suspensions of human ventricular tissue were obtained by dissociation with Ultra Turrax T5 FU (IKA-Works, Breisgau, Germany), followed by dounce homogenization. 4C templates were mixed and sequenced simultaneously in 1 HiSeq 2000 lane (Illumina, Inc., San Diego, California). Enhancer candidate regions with major and minor alleles for rs6781009 were obtained by polymerase chain reaction from human control deoxyribonucleic acid (DNA) and cloned

into the Hsp68-LacZ reporter vector. DNA was injected into the pronucleus of a fertilized Friend virus B-type strain egg, and approximately 200 injections/ construct were performed. Embryos were harvested and stained with X-gal to detect LacZ activity.

H10 cells, grown in 12-well plates in Dulbecco's Modified Eagle's medium supplemented with 10% fetal calf serum and glutamine, were transfected using polyethylenimine 25 kDa (PEI) at a 1:3 ratio (DNA:PEI). Transfections were carried out at least 3 times and measured in triplicate. Luciferase measurements were performed using a Modulus Multimode Reader luminometer (Promega Corporation, Madison, Wisconsin).

IDENTIFICATION OF CANDIDATE GENES. We considered genes to be causal candidates on the basis of: 1) the nearest gene and any other gene located within 10 kb of the sentinel SNP; 2) genes containing coding variants in linkage disequilibrium (LD) with the ST-T wave SNPs at $r^2 > 0.8$; 3) GRAIL (Gene Relationships Across Implicated Loci) analyses using the 2006 dataset to avoid confounding by subsequent GWAS discovery; and 4) genes with an expression quantitative trait locus (eQTL) analysis in cis using 4 independent sets of cardiac left ventricle and blood tissues. Ingenuity Pathway Analysis Knowledge Base March 2015 (Ingenuity Systems, Redwood City, California) was used to explore molecular pathways between proteins encoded by the 67 candidate genes from the 52 genome-wide significant loci.

We queried a *D. melanogaster* dataset containing a genome-wide phenotypic screen of cardiac-specific ribonucleic acid interference (RNAi) silencing of evolutionarily conserved genes under conditions of stress. We also queried the international database resource for the laboratory mouse (MGI [Mouse Genome Informatics]) and manually curated mammalian phenotype (MP) identifiers related to cardiac phenotypes. To illustrate that prioritized genes may play a critical role in heart development, we tested *CG4743/SLC25A26, Fhos/FHOD3, Cka/ STRN, NACα/NACA, EcR/NR1H,* and *Hand/HAND1* by performing heart-specific RNAi knockdown with the cardiac Hand4.2-Gal4 driver line.

We collected 43,278 raw Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, California) from the Gene Expression Omnibus (GEO) containing human gene expression data. A robust multichip average was used for normalization, and we subsequently conducted stringent quality control and processing of the data, which resulted in a tissue-expression matrix. After quality control, 37,427 samples remained, and we assigned 54,675 different probe sets to 19,997 different Ensembl genes used for human tissue expression profiling. To explore gene-expression of our candidate genes during cardiac differentiation, we performed ribonucleic acid sequencing using E14 Tg (Nkx2-5-EmGFP) mouse embryonic stem cells that were cultured in feeder-free conditions and subsequently differentiated.

STATISTICAL ANALYSIS. Our choice of the statistical threshold ($p < 1 \times 10^{-8}$) for the GWAS was grounded on the guidelines derived from studies of the ENCODE (Encyclopedia of DNA Elements) regions which suggests that $p < 5 \times 10^{-8}$ is the appropriate threshold for genome-wide significance in Europeans, but was also designed to provide us with additional adjustment for the multiple phenotypes tested. This threshold is conservative, considering that our 4 QRS phenotypes are also inter-related: correlation coefficients between the phenotype pairs range from r = 0.22 to 0.80. Additional details on our statistical analysis can be found in the Online Appendix.

RESULTS

Characteristics of studies, participants, genotyping arrays, and imputation are summarized in Online Tables 1 and 2. Together, our studies comprised 60,255 individuals of European ancestry ascertained in North America and Europe, with maximum sample sizes as follows: Sokolow-Lyon (n = 54,993), Cornell (n = 58,862), 12-leadsum (n = 48,632), and QRS duration (n = 60,255). Across the genome, 52 independent loci, 32 of which are novel, reached genome-wide significance for association with 1 or more QRS phenotypes (**Figure 1**, Online Figure 2, Online Table 3, Online Appendix). At each locus, we defined a single "sentinel" SNP with the lowest p value against any of the 4 phenotypes; regional association plots for the 52 loci are shown in Online Figure 3. Among the 52 loci, 32 were associated with only 1 QRS phenotype, and 20 with at least 2 phenotypes (Online Figure 4). The total number of locus-phenotype associations at $p < 10^{-8}$ was 79 (72 SNPs), of which 59 are novel (Online Table 3). Full lists of the sentinel SNPs and the SNPs associated with any phenotype at $p < 10^{-6}$ are provided in Online Tables 4 and 5. All previously known QRS duration loci showed evidence for association ($p < 10^{-6}$) (Online Table 6). Among the 32 novel loci, 8 demonstrated genome-wide significant association with Sokolow-Lyon, 9 with Cornell, 20 with 12-leadsum, and 9 with QRS duration (Online Table 5). Collectively, the total variance explained by the 52 sentinel SNPs for the QRS traits was between 2.7% (Sokolow-Lyon) and 5.0% (QRS duration) (Online Table 7). At some loci, we found evidence for

FIGURE 1 Genome-Wide Associations and Candidate Genes
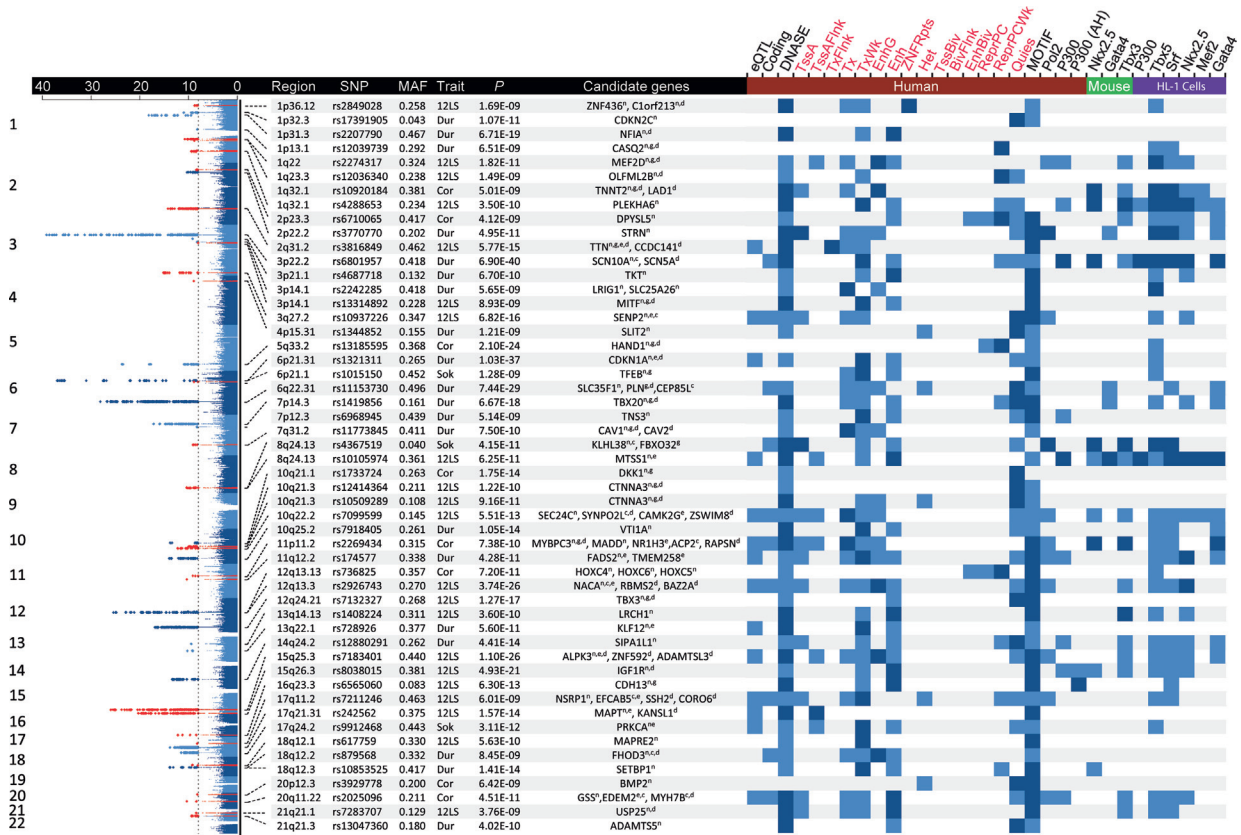
This overlay Manhattan plot shows the results for the genome-wide associations with QRS traits among Europeans. Single nucleotide polymorphisms (SNPs) reaching genome-wide significance (p < 1 × 10^{-8}) are colored **red** (novel loci) or **blue** (previously reported loci). Candidate genes have been identified by 1 or multiple strategies: n = nearest; c = coding nonsynonymous variant; g = GRAIL (Gene Relationships Across Implicated Loci) tool; e = expression quantitative trait loci (eQTL); and d = DEPICT (Data-Driven Expression-Prioritized Integration for Complex Traits) tool. The presence of associated eQTL, coding SNPs, deoxyribonuclease (DNAse) hyper-sensitivity sites, chromatin states, or transcription factor binding sites are indicated for lead SNPs **(light blue)** or those in high (r^2 > 0.8) linkage disequilibrium **(dark blue)**. 12LS = 12-lead sum product; Cor = Cornell voltage product; Dur = QRS duration; MAF = minor allele frequency; Sok = Sokolow-Lyon product.

multiple independent associations with QRS pheno-types at p < 10^{-8} in conditional analyses (7) (Online Table 8, Online Appendix). Among the 52 loci iden-tified, 8 have been associated previously with PR (reflecting atrial and atrioventricular node function), 5 with QT duration (ventricular repolarization), and 2 with heart rate (sinus node function) (Online Table 6), indicating genetic overlap among the 4 cardiac mea-sures studied. We further demonstrated that there was directional consistency of the association of common variants identified in this study with QRS phenotypes in other ethnic groups (Online Figure 1, Online Table 9, Online Appendix).

FUNCTIONAL ANNOTATION OF THE QRS ASSOCIATIONS.
To better capture common sequence variants at the 52 loci, we queried the 1000 Genomes Project dataset (8), and identified 41 nonsynonymous SNPs in 17

genes that are in high LD (r^2 > 0.8) with 12 of the sentinel SNPs (Online Table 10), representing an initial set of candidate variants that may have a functional effect on the QRS phenotypes through changes in protein structure and function.

To assess the potential role of gene expression regulation, we tested the 52 loci for enrichment of DHS (9). In an analysis across 349 diverse cell lines, cultured primary cells, and fetal tissues (10) mapped by the ENCODE project (11) and the National Institute of Health Roadmap Epigenomics Program (12), the majority (42 of 52) of sentinel SNPs were located in DHS. In human fetal heart tissue, we found that less than one-half (22 of 52) overlapped DHS, which still represents a ∼3.5-fold enrichment compared with the null expectation (p = 7.7 × 10^{-12}) **(Figure 2A)**. Further, the enrichment of genome-wide significant SNPs

FIGURE 2   Functional Annotations
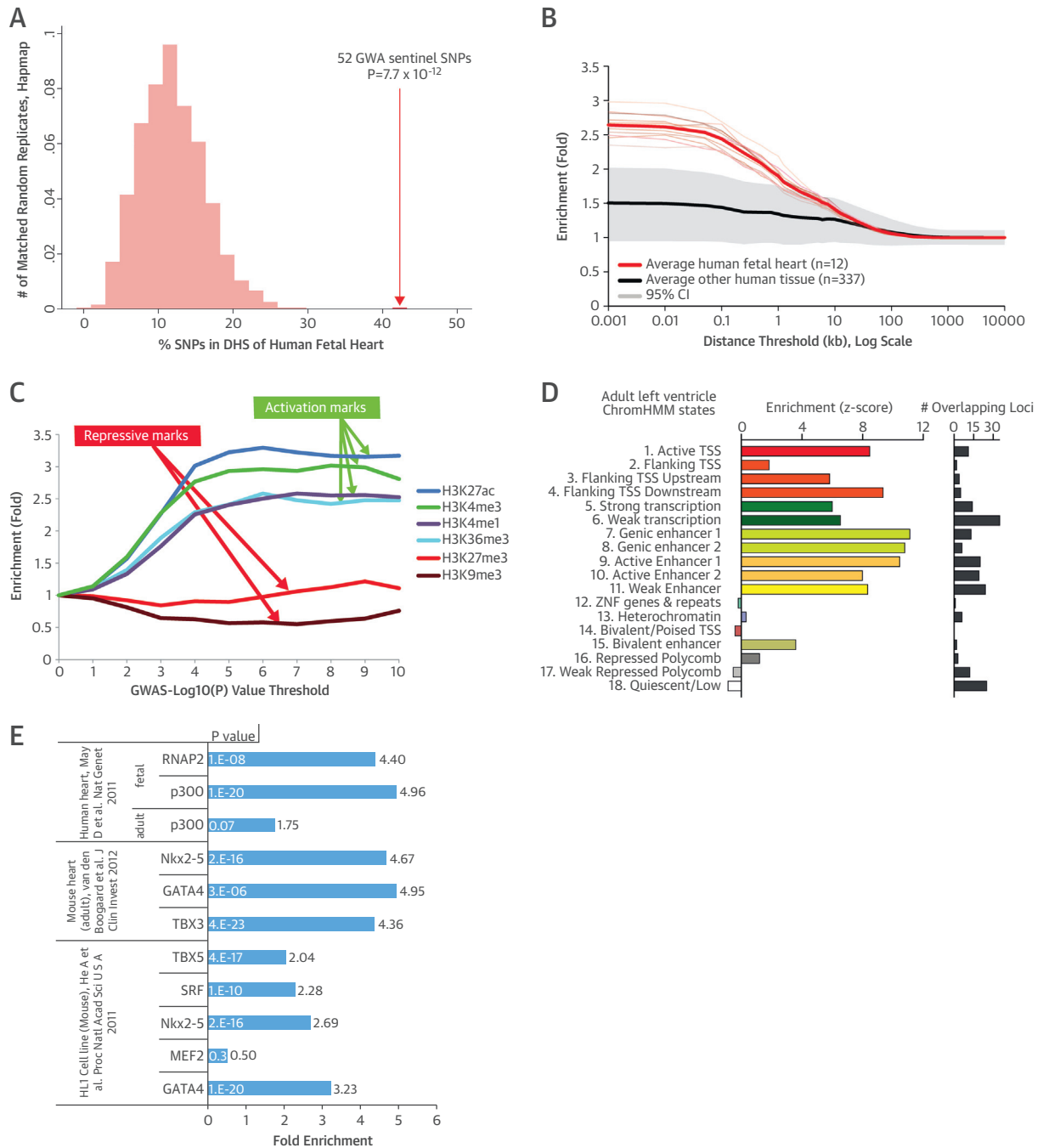
(A) The 52 sentinel SNPs are significantly enriched in deoxyribonuclease hypersensitivity sites (DHS) of the human fetal heart compared with the matched random distribution of HapMap SNPs. (B) The effect of physical distance between SNPs that meet genome-wide significance (p $< 1 \times 10^{-8}$) on enrichment of fetal heart relative to all other tissues at DHS. The enrichment is strongest at the SNP's location and decreases after 100 base pairs from the SNP sites. (C) SNPs associated with QRS traits are enriched for the activating histone modifications H3K27ac, H3K4me3, H3K4me1, and H3K36me3 in the human left ventricle, which increased at more stringent genome-wide association study (GWAS) p value thresholds. The repressive mark H3K27me3 is not enriched, whereas H3K9me3 is significantly reduced, suggesting that QRS-trait loci are predominantly expressed in the left ventricle. (D) To capture greater complexity, we performed an integrative analysis in an 18-state "expanded" ChromHMM model representative of different functional regions of the genome. The 52 loci for the 18-state model were enriched using the 6 core histone marks **(left)**; the total number of the 52 loci overlapped by each feature is shown **(right)**. (E) SNPs (p $< 1 \times 10^{-8}$) were also significantly enriched for various factors in the human heart, mouse heart, and the HL-1 cell line. CI = confidence interval; TSS = transcription start site; other abbreviations as in **Figure 1**.

($p < 10^{-8}$) in DHS was strongest within the first 100 base pairs around the sentinel variants (**Figure 2B**). Additionally, there was a strong enrichment for histone marks and chromatin states (13) associated with active enhancers, promoters, and transcription in the human heart; by contrast, no enrichment was observed for transcriptionally repressive histone marks or states (**Figures 2C and 2D**, Online Figure 5). Strikingly, we observed increasing enrichment of activating histone marks at the identified QRS loci during the process of differentiating mouse embryonic stem cells into cardiomyocytes (Online Figure 6). Altogether, these findings are consistent with earlier observations of selective enrichment of trait-associated variants within DHS of specific cells of tissue types (10), and they point to a regulatory role of the QRS-associated loci during cardiac development.

We next surveyed our genome-wide significant SNPs in DHS for perturbation of transcription factor (TF) recognition sequences, because these sites can point directly to binding events (Online Appendix). Of the 22 sentinel SNPs in human fetal heart DHS, 11 are predicted to alter TF recognition sequences (Online Table 11). When considering all genome-wide significant SNPs ($p < 10^{-8}$) as well as those in high LD ($r^2 > 0.8$), 402 SNPs in the colocalizing DHS perturb transcription recognition sequences, including those of important cardiac and muscle developmental regulators like TBX, GATA-4, and MEF2. When we intersected the GWAS results with ChIP-seq data from mouse and human cardiac tissue (14–16), we found enrichment in enhancers marked by p300, sites bound by RNA polymerase II, and the transcription factors NKX2-5, GATA-4, TBX3, TBX5, and SRF (**Figure 2E**). A total of 9 of our 52 loci contained not only fetal heart DHS but also ChIP-seq–validated TF binding sites. SNPs overlapping TF binding sites were 5.65-fold enriched within DHS ($p = 9.0 \times 10^{-10}$) but not outside of the DHS ($p = 0.20$). The associations of the 52 sentinel SNPs with all tested functional elements are summarized in **Figure 1**. We validated several candidate regulatory regions identified earlier as heart enhancers in vivo. Activity of 4 exemplar novel human cardiac enhancers in embryonic transgenic mice stained for *LacZ* enhancer reporter activity are shown in **Figure 3A**. Recently, rs6801957 (**Figure 1**) in the *SCN5A/SCN10A* locus was reported to influence the activity of a regulatory element affecting SCN5A expression (16,17). Conditional analysis (Online Table 8) revealed that rs6781009 (at 180 kb from the sentinel) is an additional novel independent signal at this locus. Our follow-up in silico and experimental results (**Figure 3**) indicate the presence of in vivo heart enhancers in genome regions associated with QRS traits.

IDENTIFICATION OF CANDIDATE GENES. Across the 52 loci, 974 annotated genes are located within 1 megabase of all sentinel SNPs. Among these genes, we prioritized potential candidates using an established complementary strategy (18,19) by choosing: 1) genes nearest to the sentinel SNP and any other genes within 10 kb (56 genes) (**Figure 1**); 2) genes containing a nonsynonymous SNP in high LD ($r^2 > 0.8$) with the sentinel SNP (11 genes) (Online Table 10); 3) protein-coding genes with *cis*-eQTL associated with sentinel SNP (14 genes) (Online Table 12); and 4) GRAIL analysis of the published data (20) (16 genes) (Online Table 13) with "cardiac," "muscle," and "heart" as the top 3 keywords describing the observed functional connections. In total, this strategy identified 67 candidate genes at the 52 loci (**Figure 1**). Pathway analysis confirmed that the list of 67 candidate genes is strongly enriched for genes known to be involved in cardiovascular and muscular system development and function ($p = 1 \times 10^{-56}$) (Online Tables 14 and 15). We have summarized the available functional annotations for all 67 candidates in Online Table 16, including established links from the Online Mendelian Inheritance in Man between candidate genes and familial cardiomyopathies (*TNNT2*, *TTN*, *PLN*, and *MYBPC3*) and cardiac arrhythmias (*CASQ2*). We also identified genes that are associated with atrial septal defects (*TBX20*) and more complex syndromes involving cardiac abnormalities such as the Schinzel-Giedion midface retraction syndrome (*SETBP1*) (21) and the ulnar-mammary syndrome (*TBX3*) (22).

INSIGHTS FROM GENE EXPRESSION PROFILING AND MODEL ORGANISMS. We explored gene expression profiles of our candidate genes in data derived from 37,427 U133 Plus 2.0 arrays (Affymetrix) across 40 annotated tissues. We could reliably assign a probe for 63 of our 67 candidate genes. On average, expression levels for these transcripts were higher in cardiac-derived samples compared with other transcripts in the same sample ($p = 9.8 \times 10^{-6}$ for heart tissue; Wilcoxon test) (Online Figure 7) and also when compared to the same transcripts in other tissues ($p = 0.005$ after Bonferroni correction) (Online Figure 8). To further investigate the potential role of these candidate genes in cardiac development, we assessed temporal gene expression patterns during in vitro differentiation of mouse embryonic stem cells via mesoderm and cardiac precursor cells to cardiomyocytes. A total of 7% of genes are mainly expressed during the embryonic stem cell stage, 22% during the mesoderm stage, 7% in the cardiac precursor stage, and 64% in the cardiomyocyte stage. Compared with other genes, the candidate genes

FIGURE 3 Functional Follow-Up of rs6781009 in the SCN5A Locus
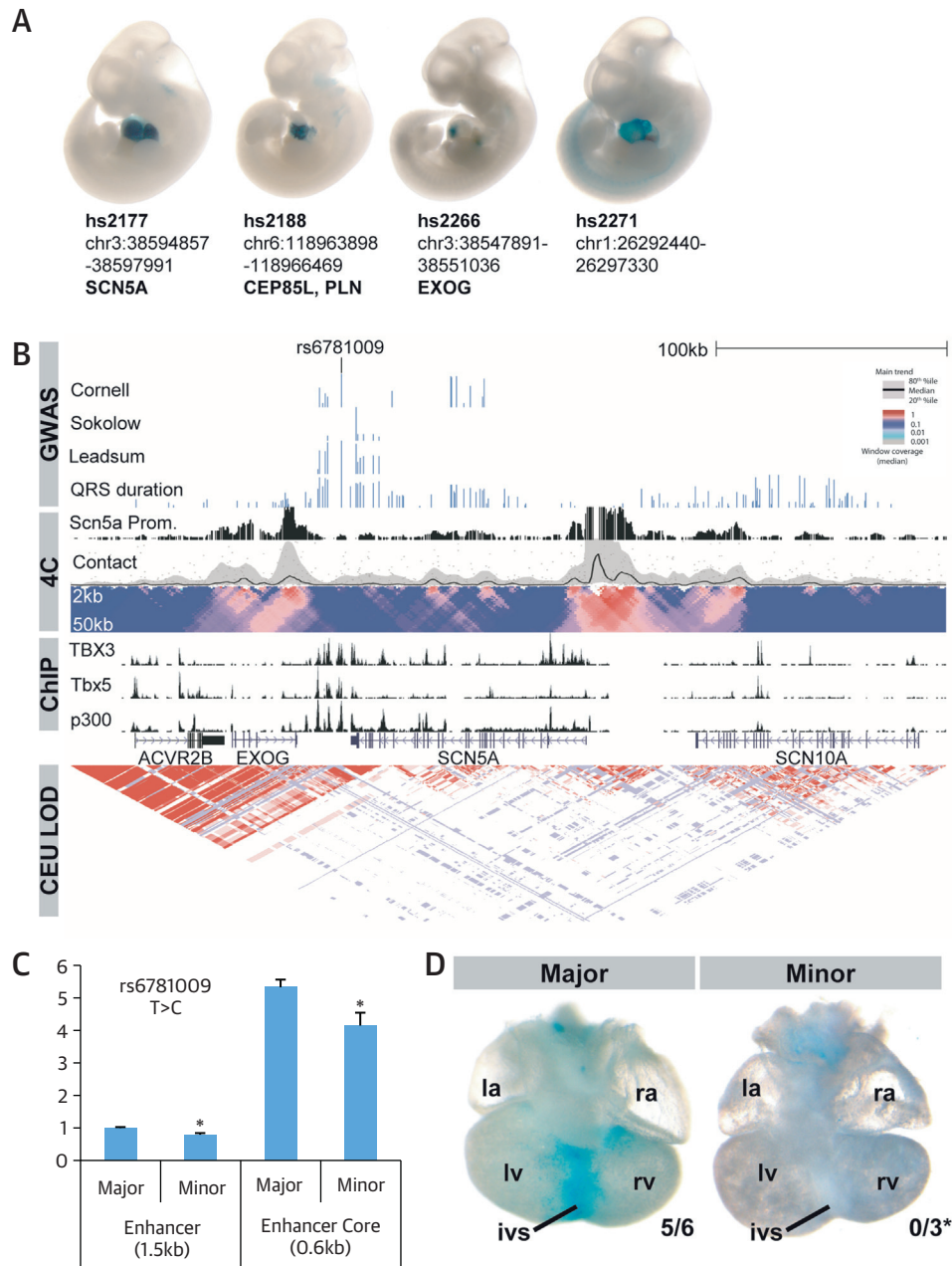
(A) In vivo activity of 4 exemplar human cardiac enhancers in embryonic transgenic mice stained for *LacZ* enhancer reporter activity **(dark blue)** are shown. (For additional examples of previously described enhancers near lead SNPs, see Online Figure 13.) **(B)** Position of the regulatory element containing rs6781009 on the SCN5A-SCN10A locus. GWAS signals are plotted on a −log(p) scale in **dark blue**. The regulatory element is bound by *TBX3*, *TBX5*, and P300 **(lower black traces)** in mice, and the contact profile of the *SCN5A* promoter obtained by 4C-seq human cardiac ventricular tissue revealed an interaction between this regulatory element and the *SCN5A* promoter **(upper black trace** and contact profile). Normalized contact intensities **(gray dots)** and their running median trends **(black line)** are depicted for the *SCN5A* promoter viewpoint. Medians are computed for 4-kb windows, and the **gray band** displays the 20% to 80% percentiles for these windows. Below the profile, statistical enrichment across differently scaled window sizes (from 2 kb **[top row]** to 50 kb **[bottom rom]**) is depicted of the observed number of sequenced ligation products over the expected total coverage of captured products, with the latter being estimated on the basis of a probabilistic background model. Local changes in color codes indicate regions that are statistically enriched for captured sequences. The **lowest box** shows the linkage disequilibrium pattern for the HapMap CEU population. **(C)** Luciferase assay performed in H10 cells showing a high constitutive activity for the enhancer core element (0.6 kb) containing the major allele for rs6781009, which is reduced for the minor allele in both a large enhancer construct (1.5 kb), as well as in the core enhancer element (0.6 kb). *p < 0.01. **(D)** Dorsal views of hearts containing the human regulatory element with the major versus minor allele for rs6781009 in a *LacZ* reporter vector, showing specific expression of the enhancer in the interventricular septum (ivs) for the major allele, which is absent for the minor allele. *p < 0.05. la = left atrium; lv = left ventricle; ra = right atrium; rv = right ventricle; other abbreviations as in **Figure 1**.

were more highly expressed in cardiomyocytes ($p = 5.4 \times 10^{-8}$; Wilcoxon test) (Online Figure 9). These results suggest that our candidate gene set was enriched for genes that were differentially expressed in cardiac tissue and increasingly expressed during cardiac development.

Next, we analyzed data from model organisms to explore the function of the selected candidate genes. From cardiac tissue-specific RNAi knockdown data collected in *D. melanogaster*, we found that the 67 candidate genes were 2.3-fold enriched for stress-induced cardiac death (9 genes; $p = 1.84 \times 10^{-2}$) (Online Figure 10). To illustrate that prioritized genes may play a critical role in heart development, we tested 4 (*CG4743/SLC25A26, Fhos/FHOD3, Cka/STRN,* and *NACα/NACA*) of these 9 genes with unknown cardiac function by performing heart-specific RNAi knockdown with the cardiac Hand4.2-Gal4 driver line. We also retested *EcR/NR1H*, which has multiple homologous genes in mammals, as well as *Hand/HAND1*, as this gene was only tested as a full-knockout in early development but not in the adult *D. melanogaster* heart using cardiac-specific knockdown. Adult hearts of Cka/STRN, *NACα/NACA,* and *EcR*/NR1H RNAi showed severe cardiac defects (**Figure 4**). Knockdown of *Hand*/HAND1 and *Cka*/STRN both had a reduced cardiac heart rate. We also expanded on gene-by-gene analysis and identified 6 further genes causing cardiac abnormalities (Online Appendix, Online Table 17). From the Mouse Genome Informatics database, knockout models were annotated for 45 orthologues of the 67 candidate genes, of which 18 (40%) revealed a cardiac pheno-type (Online Table 16). This represents a 5.2-fold enrichment compared with randomly matched sets of 67 genes ($p = 3.4 \times 10^{-14}$) (Online Figure 10). Given the evolutionary conservation, the observed heart phenotypes in these model organisms suggest potentially important roles for the significant GWAS loci in electrical and contractile properties of the human heart.

Interestingly, the 11p11.2 locus harbors multiple candidate genes (**Figure 1**), including *MYBPC3, ACP2, MADD,* and *NR1H3. MYBPC3* deficiency is well established to cause hypertrophic and dilated cardiomy-opathies in both human and mouse models and, thus, represents a plausible candidate gene (Online Table 16). In addition to *MYBPC3,* eQTL and histone modification data also suggest a potential role for *NR1H3* (Online Figure 11), as decreased expression of *NR1H3* was associated with higher QRS voltages. However, *NR1H3*-deficient mice do not spontane-ously develop a cardiac hypertrophic phenotype (MGI: 1352462). To study the potential cardiac effects of *NR1H3,* we created a transgenic mouse with cardiac-specific overexpression of *NR1H3* under the control of the *Myh6* promoter and found a diminished susceptibility to perturbations such as transverse aortic constriction and angiotensin II infusion that provoke cardiac hypertrophy (23). This observation is in line with protective effects due to treatment with T0901317, a synthetic *NR1H3* agonist, in mice chal-lenged with aortic constriction (24). These data highlight the importance of systematic approaches to identify causal genes beyond well-known candidates.

INSIGHTS FROM DEPICT. As a complementary approach, we employed the newly developed computational tool DEPICT (Data-Driven Expression-Prioritized Integration for Complex Traits) (25) to analyze functional connections among associated loci (Online Appendix). Enrichment of expression in 209 particular tissues and cell types identified heart and heart ventricles as the most relevant tissue for our association findings (**Figure 5A**, Online Table 18) and identified 404 significantly (false discovery rate <5%) enriched gene sets (Online Table 19). Comparing the names of these sets with those of the remaining 14,057 gene sets showed an over-representation of the common key words "abnormal," "muscle," "heart," "cardiac," and "morphology" (Online Table 20). We investigated similarities among gene sets by clustering them on the basis of the correlation between scores for all genes (Online Appendix). Many of the resulting 43 meta-gene sets were correlated and relevant to cardiac biology (**Figure 5B**). As an example, we showed the correlation structure within the second most significant meta-gene set "dilated heart left ventricle" (Online Figure 12). When priori-tizing genes on the basis of functional similarities among genes from different associated regions, DEPICT identified 35 genes (false discovery rate <5%) at 27 of the 52 loci (**Figure 1**, Online Table 21).

DISCUSSION

In this study, we performed a meta-analysis of GWAS in 73,518 individuals for 4 quantitative QRS pheno-types and identified 52 independent genetic loci influencing these traits with 79 locus-phenotype associations; the majority of these discoveries are novel. Our loci were colocalized with open chromatin, histone modification, and TF binding sites, specif-ically in cardiac tissue, and contain in vivo functional enhancers. We also provided direct evidence that rs6781009, located in a cardiac enhancer, interacts with the promoter of SCN5A to modify expression levels. On the basis of multiple criteria, we defined a core set of 67 candidate genes that we believe are

FIGURE 4   Heart-Specific RNAi Knockdown in *Drosophila*
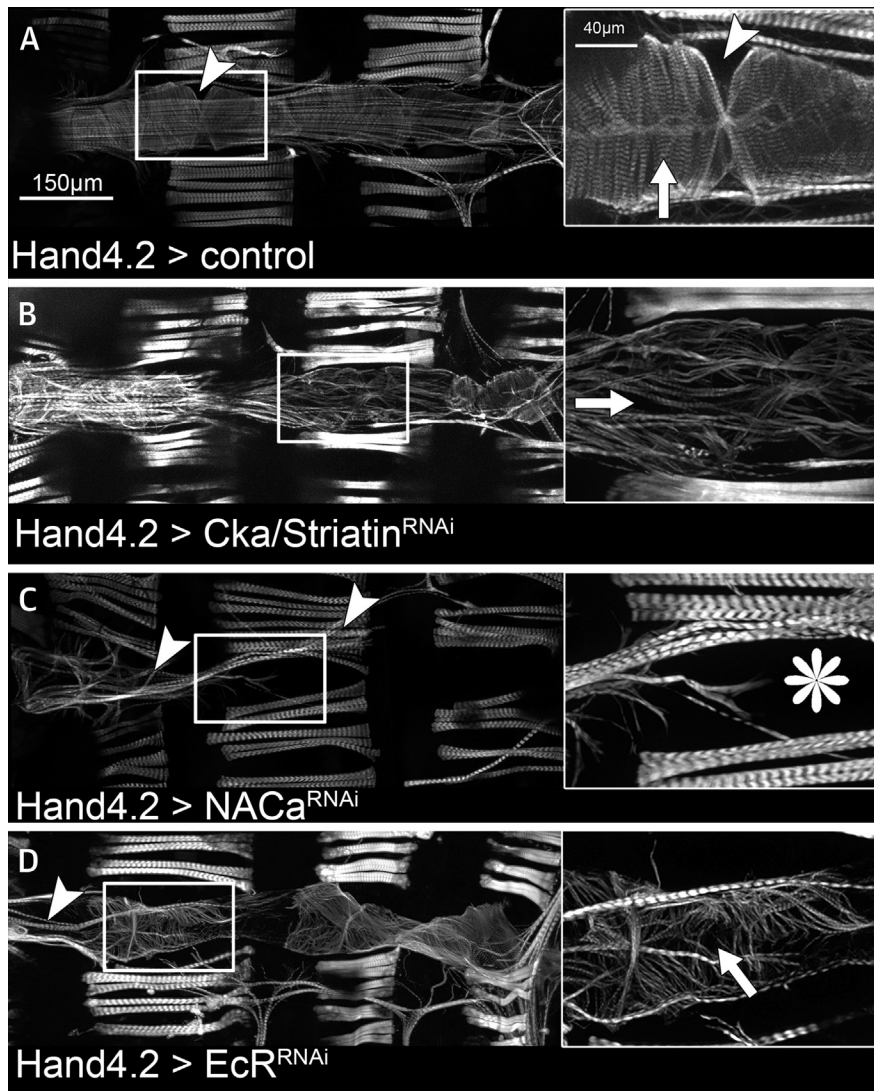
Cardiac defects upon heart-specific ribonucleic acid interference (RNAi) knockdown are seen in *Drosophila*. **(A)** Wild-type dorsal heart tube stained with the F-actin stain phalloidin. The magnified region **(right)** is highlighted. **Arrowheads** point to ostia (inflow tracks), and the **arrow** shows the circumferential orientation of myofibrils. **(B)** Cka/Striatin RNAi induces myofibrillar disarrangement. Myofibrils are oriented in a disorganized, mainly anterior-posterior orientation with gaps in between **(arrow)**. **(C)** Knockdown of NACα/NACA causes severe cardiac tissue disintegration. Adult cardiomyocyte tissue may be completely absent **(asterisk)**, whereas some heart-associated longitudinal muscles are still present **(arrowheads)**. At larval stages, the heart is much less affected, suggesting a maturation or remodeling defect. **(D)** Knockdown of EcR/NR1H blocks cardiac remodeling and causes myofibrillar disarray **(arrow)**. Ventral longitudinal muscles are also abnormal **(arrowhead)**.

likely to influence cardiac mass and function. We have provided several exemplar experiments to further support this hypothesis.

We identified a number of loci containing genes that are directly or indirectly key to the function of cardiomyocytes and cardiac function (**Central Illustration**). *TTN, MYBPC3, TNNT2, SYNPO2L,* and *MYH7B* are essential components of the cardiac sarcomere; *PLN, CTNNA3, PRKCA, CASQ2,* and *STRN* are also examples of genes essential for cardiac myocyte function; whereas several key cardiac transcription factors are prominently involved in cardiac muscle and tissue development, such as *MEF2D, HAND1, TBX20, TBX3,* and *NACA*. The abundance of

FIGURE 5   Functional Connections of Gene Expression Networks
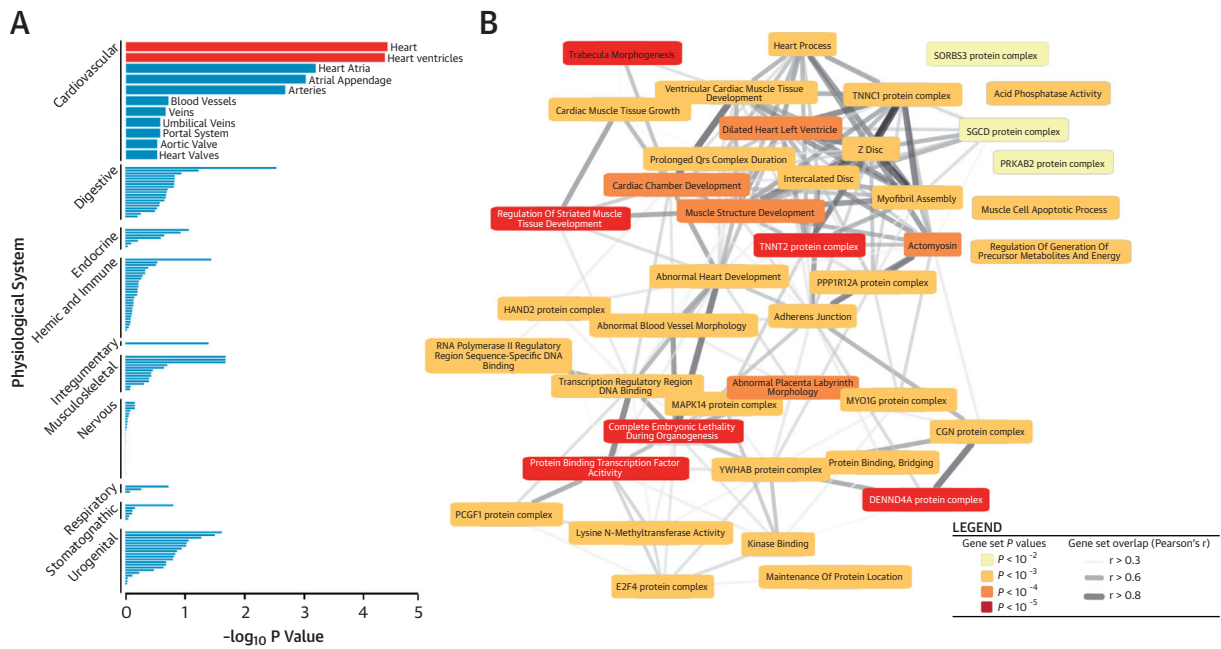
In the DEPICT (Data-Driven Expression-Prioritized Integration for Complex Traits) analysis, **(A)** plots show the enrichment of loci associated with QRS traits in specific physiological systems. **(B)** In a graphic display of DEPICT gene set enrichment analysis, meta-gene sets are represented by nodes colored according to statistical significance, and similarities between them are indicated by edges scaled according to their correlation (only correlations with r > 0.3 are shown).
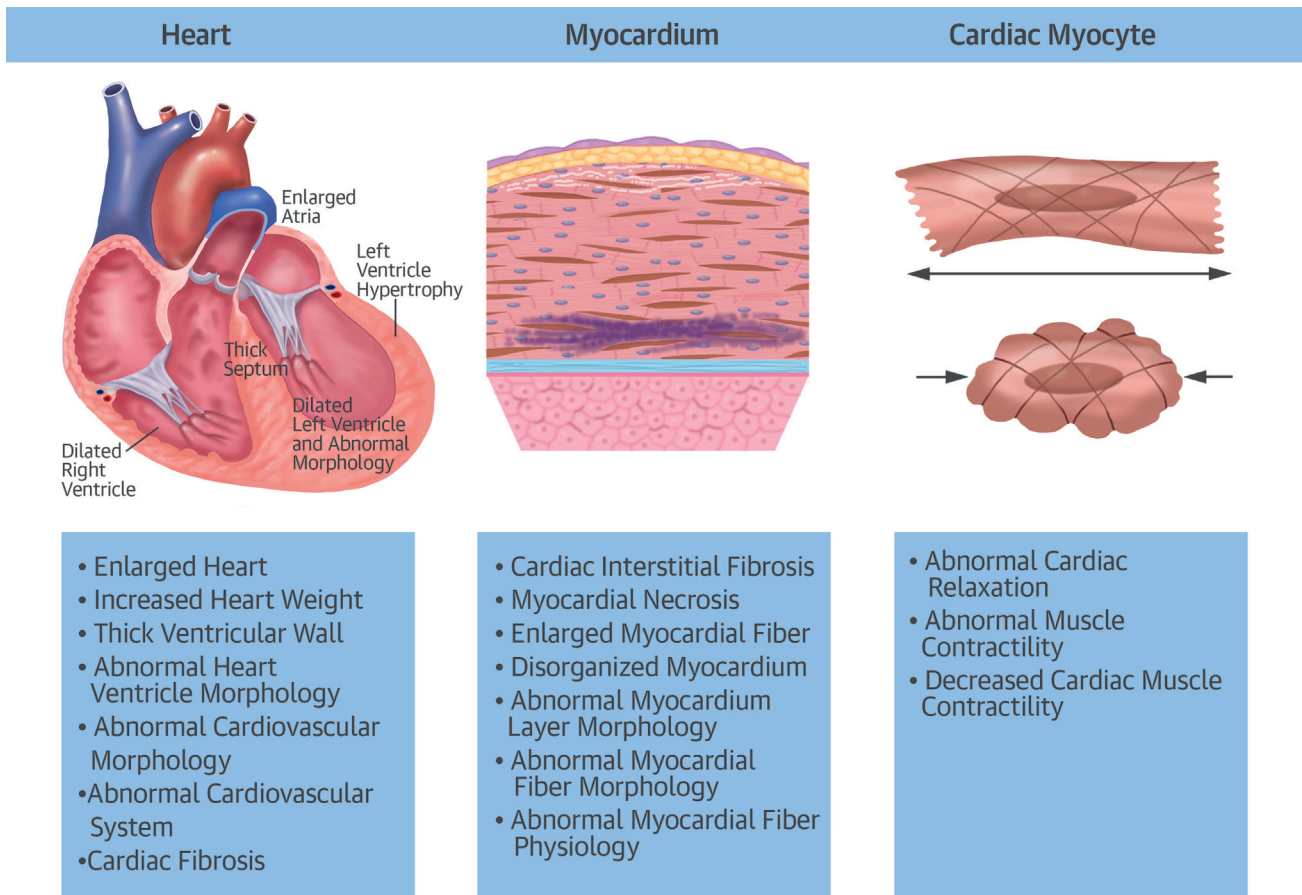
candidate genes known to be involved in cardiac muscle function strengthens the hypothesis that the easily obtainable QRS-voltage phenotypes of the ECG are effective in capturing unknown loci that harbor genes likely to play an important role in left ventricular mass, but these are currently not well understood. The colocalization of our genetic loci with regulatory DNA elements (e.g., enhancers, promoters, and transcription factor binding sites) that are active in cardiac tissues further supports the relevance of the genes within these loci. The current work was not designed to provide an explanation for association of each loci and each individual gene.

Clearly, future translational efforts should be undertaken to resolve the causal genes and exact molecular machinery resulting in the phenotype and should consider mapping the effect of genetic variants on these functional elements at each of the identified loci. Nevertheless, we have provided some exemplar preliminary elements to offer early insights into strategies that can be undertaken to follow-up our findings. For example, we performed a series of experiments to demonstrate in vivo effects of rs6781009 on expression. Dedicated experiments

might also elucidate loci containing effects on multiple plausible genes. In 1 of our loci, we identified a very strong candidate gene (*MYBPC3*) that is well known to be involved in hypertrophic cardiomyopathies. However, using additional layers of information derived from gene expression and histone modifications, we also considered *NR1H3* and were able to link overexpression of this gene to cardiac protection of hypertrophy. These examples fuel our expectation that the presented shortlist of SNP associations and the identified candidate genes provided in this work are valuable resources that will help to prioritize and guide future translational studies to further our knowledge on the (patho)physiology of cardiac hypertrophy.

STUDY LIMITATIONS. As in all current GWAS, we have only studied a finite number (~2.8 million) of markers on the genome. Additional fine-mapping studies might be required to narrow the signal of association even further and to identify the potential causal variants with higher accuracy. Also, additional exome-focused arrays or whole-genome sequencing might lead to a stronger signal within a locus or to multiple additional

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CENTRAL ILLUSTRATION Gene-Related Cardiac Conditions

Heart	Myocardium	Cardiac Myocyte

Enlarged Atria

Left Ventricle Hypertrophy

Thick Septum

Dilated Left Ventricle and Abnormal Morphology

Dilated Right Ventricle

- Enlarged Heart
- Increased Heart Weight
- Thick Ventricular Wall
- Abnormal Heart Ventricle Morphology
- Abnormal Cardiovascular Morphology
- Abnormal Cardiovascular System
- Cardiac Fibrosis

- Cardiac Interstitial Fibrosis
- Myocardial Necrosis
- Enlarged Myocardial Fiber
- Disorganized Myocardium
- Abnormal Myocardium Layer Morphology
- Abnormal Myocardial Fiber Morphology
- Abnormal Myocardial Fiber Physiology

- Abnormal Cardiac Relaxation
- Abnormal Muscle Contractility
- Decreased Cardiac Muscle Contractility

van der Harst, P. et al. J Am Coll Cardiol. 2016;68(13):1435–48.

In this study, a number of gene-containing loci were identified that influence a variety of abnormalities in the dysfunctional heart, myocardium, and cardiac myocytes. Analyzing the QRS genome-wide association study results using the DEPICT (Data-Driven Expression-Prioritized Integration for Complex Traits) tool identified 43 meta-gene sets (Figure 5B); 1 of these is the "dilated heart left ventricle," of which the effects of the individual gene sets are visualized in the illustration. The correlation substructure and the p values of this gene set are also displayed in Online Figure 12.

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independent signals within a locus. To understand genetic mechanisms and to identify candidate genes, we have studied eQTLs. Although we studied the largest set of human cardiac eQTLs available to date, the absolute number of studied samples is relatively small compared to eQTL data available in easily accessible peripheral blood. Finally, our ECG indexes are generally considered to be markers of cardiac hypertrophy; they also might reflect electrical remodeling of the action potential and not mass per se. Nevertheless, the variables studied here harbor important prognostic information, independently

from cardiac mass parameters as assessed by echocardiography (26). This further underscores the relevance of the trait studied and the importance of understanding its genetic determinants.

CONCLUSIONS

In this study, we identified 52 genomic loci, of which 32 are novel and associated with electrically active cardiac mass. We prioritized 67 candidate genes and showed their relevance in cardiac biology using bioinformatic approaches, and we performed in vitro and

in vivo experiments, going beyond the classical GWAS approach. To facilitate and accelerate future studies aimed at a better understanding of cardiac hypertrophy, heart failure, and related diseases, we made our results of genome-wide associations publicly available.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Numerous genetic loci have been identified that are associated with electrocardiographic markers of ventricular hypertrophy.

TRANSLATIONAL OUTLOOK: A better understanding of the genetic pathways underlying increased myocardial mass could be used to target therapeutic interventions that improve clinical outcomes for patients with hypertension, heart failure, and various forms of congenital heart disease.

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KEY WORDS electrocardiogram, genetic association study, heart failure, left ventricular hypertrophy, QRS

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this article.