Fifty-two Genetic Loci Influencing Myocardial Mass

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Affiliations: are provided at the end of the manuscript

Word count: 4978

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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.

OBJECTIVE: 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, reliably associated with one or more QRS phenotypes at $P<1\times10^{-8}$. 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 provide evidence that these loci play a role in cardiac hypertrophy. We further highlight 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.

Keywords: Genetic association study, QRS, left ventricular hypertrophy, heart failure, electrocardiogram, genes

Abbreviations list

DHS	Deoxyribonuclease hypersensitivity sites
DNase	Deoxyribonuclease
ECG	Electrocardiogram
eQTL	Expression quantitative trait locus
FDR	False Discovery Rate
GWAS	Genome-wide association study
LD	Linkage Disequilibrium
RNAi	Ribonucleic acid interference
SNP	Single Nucleotide Polymorphisms
TF	Transcription Factor

Introduction

The role of the heart is to provide adequate circulation of blood to meet the body's requirements of oxygen and nutrients. The QRS complex on the ECG is 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 the 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 preclinical cardiovascular diseases such as cardiac hypertrophy, heart failure, and various cardiomyopathies, and can also predict cardiovascular mortality(3-6).

Identification of specific genes influencing the QRS complex may thus enhance our understanding of the human heart and ultimately lead to prevention of cardiovascular disease and death. To further our understanding of the genetic factors influencing the QRS complex, we carried out a large scale 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 on the methods can be found in the **Supplementary Note**.

Genome wide analyses and replication testing

Our study design is summarized in **Fig. S1**. Briefly, we combined summary statistics from 24 studies for up to 2,766,983 autosomal SNPs using an inverse-variance fixed-effects meta-analysis for each QRS trait. We performed replication testing for loci showing suggestive association ($1\times10^{-8}<P<5\times10^{-7}$) (**table S1 and S2**). The threshold for genome-wide significance was set at $P<1\times10^{-8}$.

DNA Functional elements, coding variation and enrichment analyses

We performed an intersection between SNPs and regions of DHSs, 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.

Experimental cardiac enhancer studies

Single cell suspensions of human ventricular tissue was obtained by dissociation with IKA Ultra Turrax T5 FU, followed by dounce homogenization. 4C templates were mixed and sequenced simultaneously in one Illumina HiSeq 2000 lane. Enhancer candidate regions with major and minor allele for rs6781009 were obtained by PCR from human control DNA and cloned into the Hsp68-LacZ reporter vector. DNA was injected into the pronucleus of fertilized FVB strain egg and approximately 200 injections per construct were performed. Embryos were harvested, stained with X-gal to detect LacZ activity.

H10 cells, grown in 12-well plates in DMEM supplemented with 10% FCS (Gibco-BRL) and glutamine, were transfected using polyethylenimine 25 kDa (PEI, Brunschwick) at a 1:3 ratio (DNA:PEI). Transfections were carried out at least three times and measured in triplo. Luciferase measurements were performed using a Promega Turner Biosystems Modulus Multimode Reader luminometer.

Identification of candidate genes

We considered genes to be causal candidates based on 1) the nearest gene and any other gene located within 10kb of the sentinel SNP; 2) genes containing coding variants in LD with the ST-T wave SNPs at r^2>0.8; 3) GRAIL analyses using the 2006 dataset to avoid confounding by subsequent GWAS discovery, and 3) genes with an eQTL analyses in cis using 4 independent sets of cardiac left ventricle and blood tissues. Ingenuity Pathway Analysis (IPA) Knowledge Base March 2015 (Ingenuity Systems, CA, USA) was used to explore molecular pathways between proteins encoded by the 67 candidate genes from the 52 genome-wide significant loci.

Drosophila melanogaster and Mus Musculus methods

We queried a *D. melanogaster* dataset containing a genome-wide phenotypic screen of cardiac specific 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 Phenotypes (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.

Gene expression profiling and cardiomyocyte differentiation analysis

We collected 43,278 raw Affymetrix Human Genome U133 Plus 2.0 Arrays from the Gene Expression Omnibus (GEO) containing human gene expression data. RMA was used for normalization and 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 assigned 54,675 different probesets 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 RNA-sequencing using E14 Tg(Nkx2-5-EmGFP) mouse embryonic stem cells cultured feeder-free conditions and subsequently differentiated.

Results

Large scale meta-analysis of genome wide association studies

Characteristics of studies, participants, genotyping arrays and imputation are summarized in **table S1 and S2**. Together our studies comprise 60,255 individuals of European ancestry ascertained in North America and Europe, with a maximum sample size of 54,993 for Sokolow-Lyon, 58,862 for Cornell, 48,632 for 12-leadsum, and 60,255 for QRS duration. Across the genome, 52 independent loci, 32 of which are novel, reached genome-wide significance for association with one or more QRS phenotypes (**Fig. 1**, **Fig. S2, table S3** and **Supplementary Note**). At each locus, we defined a single 'sentinel' SNP with the lowest *P*-value against any of the four phenotypes; regional association plots for the 52 loci are shown in **Fig. S3**. Among the 52 loci, 32 were associated with only one QRS phenotype, and 20 with at least two phenotypes (**Fig. S4)**. The total number of locus-phenotype associations at $P<10^{-8}$ was 79 (72 SNPs), of which 59 are novel (**table S3**). Full lists of the sentinel SNPs and the SNPs associated with any phenotype at $P<10^{-6}$ are provided in **table S4 and S5**. All previously known QRS duration loci showed evidence for association ($P<10^{-6}$, **table S6**). 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 (**table S5**). 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) (**table S7**). At some loci we found evidence for multiple independent associations with QRS phenotypes at $P<10^{-8}$ in conditional analyses(7) (**table S8** and **Supplementary Note**). Among the 52 loci identified 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) (**table S6**), indicating genetic overlap among the four cardiac measures 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 (**Fig. S1, table S9,** and **Supplementary note**).

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 non-synonymous SNPs in 17 genes that are in high LD ($r^2>0.8$) with 12 of the sentinel SNPs (**table S10**), which represent 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 deoxyribonuclease I (DNase I) hypersensitive sites (DHSs)(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 DHSs. In human fetal heart tissue we found that less than half (22 of 52) overlapped DHSs, which still represents a ~3.5-fold enrichment compared to the null expectation ($P=7.7\times10^{-12}$, **Fig. 2A**). Further, the enrichment of genome-wide significant SNPs ($P<10^{-8}$) in DHSs was strongest within the first 100 bp around the sentinel variants (**Fig. 2B**). In addition, there was a strong enrichment for histone marks and chromatin states(13) associated with active enhancers, promoters and transcription in human heart; by contrast no enrichment was observed for transcriptionally repressive histone marks or states (**Fig. 2, C and D and Fig. S5**). 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 (**Fig. S6**). Altogether, these findings are consistent with earlier observations of selective enrichment of trait-associated variants within DHSs of specific cell of tissue types(10), and point to a regulatory role of the QRS-associated loci during cardiac development.

We next surveyed our genome-wide significant SNPs in DHSs for perturbation of transcription factor (TF) recognition sequences, since these sites can point directly to binding events (**Supplementary Note**). Of the 22 sentinel SNPs in human fetal heart DHSs, 11 are predicted to alter TF recognition sequences (**table S11**). 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 co-localizing DHSs 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 (RNAP2), and the transcription factors NKX2-5, GATA-4, TBX3, TBX5, and SRF (**Fig. 2E**). Nine of our 52 loci contained not only fetal heart DHSs but also ChIP-seq validated TF binding sites. SNPs overlapping TF binding sites were 5.65 fold enriched within DHSs ($P=9.0\times10^{-10}$) but not outside DHSs ($P=0.20$). The associations of the 52 sentinel SNPs with all tested functional elements are summarized in **Fig. 1**. We validated several candidate regulatory regions identified above 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 **Fig. 3A**. Recently, rs6801957 (**Fig. 1**) in the *SCN5A/SCN10A* locus was reported to influence the activity of a regulatory element affecting SCN5A expression(16,17). Conditional analysis (**table S8**) 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 (**Fig. 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 Mb of all sentinel SNPs. Among these genes, we prioritized potential candidates using an established complementary strategy (18,19); we chose (i.) Genes nearest to the sentinel SNP, and any other genes within 10kb (56 genes; **Fig. 1**); (ii.) Genes containing a non-synonymous SNP in high LD ($r^2>0.8$) with the sentinel SNP (11 genes; **table S10**); (iii.) Protein-coding genes with *cis*-eQTL associated with sentinel SNP (14 genes; **table S12**), and (iv.) GRAIL literature analysis(20) (16 genes **table S13**) 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 (**Fig. 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=1×10^{-56}; **table S14 and S15**). We have summarized the available functional annotations for all 67 candidates in **table S16**, including established links from the Online Mendelian Inheritance in Man (OMIM) between candidate genes and familial cardiomyopathies (*TNNT2*, *TTN*, *PLN*, *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 Affymetrix U133 Plus 2.0 arrays 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 to other transcripts in the same sample (P=9.8×10^{-6} for heart tissue; Wilcoxon test; **Fig. S7**) and also when compared to the same transcripts in other tissues (P=0.005 after Bonferroni correction; **Fig. S8**). 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 (ESC) via mesoderm (MD) and cardiac precursor (CP) cells to cardiomyocytes (CM). Seven percent of genes are mainly expressed during the ESC stage, 22% during MD stage, 7% in the CP stage and 64% in the cardiomyocyte stage. Compared to other genes, the candidate genes were more highly expressed in cardiomyocytes (P=5.4×10^{-8}, Wilcoxon test; **Fig. S9**). These results suggest that our candidate gene set is enriched for genes 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=1.84×10^{-2}; **Fig. S10**). To illustrate that prioritized genes may play a critical role in heart development we tested 4 (*CG4743/SLC25A26, Fhos/FHOD3, Cka/STRN, 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 re-tested *EcR/NR1H*, which has multiple homologous genes in mammals, as well as *Hand*/HAND1 as this gene was only tested in as a full-knockout in early development but not in adult *D. melanogaster* heart using cardiac specific knockdown. Adult hearts of Cka/STRN, *NACα*/NACA, and *EcR*/NR1H

RNAi showed severe cardiac defects (**Fig. 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 (**Supplementary Note** and **table S17**). 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 phenotype (**table S16**). This represents a 5.2-fold enrichment compared to randomly matched sets of 67 genes (P=3.4×10^{-14}; **Fig. S10**). 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 (**Fig. 1**), including *MYBPC3*, *ACP2*, *MADD*, and *NR1H3*. *MYBPC3* deficiency is well established to cause hypertrophic and dilated cardiomyopathies in both human and mouse models and thus represents a plausible candidate gene (**table S16**). In addition to *MYBPC3*, eQTL and histone modification data also suggests a potential role for *NR1H3* (**Fig. S11**), as decreased expression of *NR1H3* was associated with higher QRS voltages. However, *NR1H3* deficient mice do not spontaneously 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 challenged with aortic constriction(24). These data highlight the importance of systematic approaches to identify causal genes beyond well-known candidates.

Insights from Data-Driven Expression-Prioritized Integration for Complex Traits (DEPICT)

As a complementary approach we employed the newly developed computational tool DEPICT(25) to analyze functional connections among associated loci (**Supplementary Note**). Enrichment of expression in 209 particular tissues and cell types identified heart and heart ventricles as the most relevant tissue for our association findings (**Fig. 5A; table S18**) and identified 404 significantly (FDR <5%) enriched gene sets (**table S19**). 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', 'Morphology' (**table S20**). We investigated similarities among gene sets by clustering them on the basis

of the correlation between scores for all genes (**Supplementary Note**). Many of the resulting 43 meta-gene sets are correlated and relevant to cardiac biology (**Fig. 5B**). As an example we show the correlation structure within the second most significant meta-gene set "Dilated Heart Left Ventricle" (**Fig. S12**). When prioritizing genes based on functional similarities among genes from different associated regions DEPICT identified 35 genes (FDR<5%) at 27 of the 52 loci (**Fig. 1, table S21**).

Discussion

In this study, we performed a meta-analysis of GWAS in 73,518 individuals for 4 quantitative QRS phenotypes and identified 52 independent genetic loci influencing these traits with 79 locus-phenotype associations; the majority of these discoveries are novel. Our loci are co-localized with open chromatin, histone modification, and TF binding sites specifically in cardiac tissue, and contain *in vivo* functional enhancers. We also provide direct evidence that rs6781009, located in a cardiac enhancer, interacts with the promoter of SCN5A to modify expression levels. Based on multiple criteria, we defined a core set of 67 candidate genes which we believe are 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 the function of cardiomyocytes and cardiac function. *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; while 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 candidate genes known to be involved in cardiac muscle function strengthen the hypothesis that the easily obtainable QRS-voltage phenotypes of the electrocardiogram are effective in capturing unknown loci that harbor genes that are likely to play an important role in left ventricular mass but are currently not well understood. The co-localization of our genetic loci with regulatory DNA elements (e.g. enhancers, promoters and transcription factor binding sites) that are active in cardiac tissues further support 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. It is clear that future translational efforts should be undertaken to resolve the causal genes and exact molecular machinery resulting the in the phenotype should consider mapping effect of genetic variants

on the these functional elements at each of the identified loci. Nevertheless we have provided some exemplar preliminary elements to provide some early insights in 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 one of our loci we identified a very strong candidate gene (*MYBPC3*), well known to be involved in hypertrophic cardiomyopaties. 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 a valuable resource that will help to prioritize and guide future translational studies to further our knowledge on the (patho)physiology of cardiac hypertrophy.

Our findings do have some limitations that warrant consideration. As for all current GWAS, we have only studied a finite number (~2.8 million) marker on the genome. Further 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 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 eQTL available to date, the absolute number of studied samples is relatively small compared to eQTL data available in easily accessible peripheral blood. Finally, our electrocardiographic indices are generally considered markers of cardiac hypertrophy, they may also 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.

In conclusion, we have identified 52 genomic loci, of which 32 are novel, associated with electrically active cardiac mass, prioritized 67 candidate genes and showed their relevance in cardiac biology using bioinformatics approaches and 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.

Perspectives

COMPETENCY IN MEDICAL KNOWLEDGE: Abnormalities of cardiac mass are underlying many cardiovascular diseases such as heart failure. The lack of knowledge surrounding the basis of cardiomyocyte dysfunction and heart failure susceptibility is a major roadblock to understand risk for heart failure and to designing innovative strategies for therapy.

TRANSLATIONAL OUTLOOK: These findings will be a valuable resource for studying biological processes underlying cardiac mass, ultimately leading to prevention of cardiovascular disease and death.

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Acknowledgements

A detailed list of acknowledgements is provided in the **Supplementary Information**.

Author contributions

See **Supplementary Information**

Figure legends

Fig. 1 Overlay Manhattan plot showing the results for the genome-wide associations with QRS traits amongst Europeans. SNPs reaching genome-wide significance ($P<1x10^{-8}$) are colored red (novel loci) or blue (previously reported loci). Candidate genes have been identified by one or multiple strategies; [n] nearest; [c] coding non-synonymous variant; [g] GRAIL tool; [e] eQTL; [d] DEPICT tool. The presence of associated eQTL, coding SNPs, DNAse hypersensitivity sites, chromatin states, TF binding sites are indicated for lead SNPs (light blue) or those in high ($r^2>0.8$) LD (dark blue).

Fig. 2 (**a**) The 52 sentinel SNPs are significantly enriched in DHSs of the human fetal heart compared to the matched random distribution of HapMap SNPs. (**b**) The impact of physical distance between SNPs that meet genome wide significance ($P<1\times10^{-8}$) on enrichment of fetal heart relative to all other tissues at DHs. The enrichment is strongest at the SNP's location and decreases after 100bp from the SNP sites. (**c**) SNPs associated with QRS traits are enriched for the activating histone modifications H3K27ac, H3K4me3, H3K4me1 and H3K36me3 in human left ventricle, which increased at more stringent GWAS *P*-value thresholds. The repressive mark H3K27me3 is not enriched while H3K9me3 is significantly reduced, suggesting that QRS-trait loci are predominantly expressed in the left ventricle. (**d**) To capture the greater complexity we performed an integrative analysis in an 18-state 'expanded' ChromHMM model representative of different functional regions of the genome. The left panel shows the enrichment of the 52 loci for the 18-state model using the six core histone marks. The right panel shows the total number of the 52 loci overlapped by each feature. (**e**) SNPs ($P<1\times10^{-8}$) were also significantly enriched for various factors in the human heart, mouse heart and the HL-1 cell-line.

Fig. 3 (**a**) *In vivo* activity of 4 exemplar human cardiac enhancers in embryonic transgenic mice stained for *LacZ* enhancer reporter activity (dark blue). Additional examples of previously described enhancers near lead SNPs are provided in **Fig. S13** (**b**) Position of the regulatory element containing rs6781009 on the SCN5A-SCN10A locus. GWAS signals are plotted on -log(*P*) scale in dark blue. The regulatory element is bound by *TBX3*, *TBX5*, and P300 (lower black traces) in mouse, 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 20-80% percentiles for these windows. Below the profile statistical enrichment across differently scaled window sizes (from 2 kb (top row) to 50 kb (bottom)) is depicted of the observed number of sequenced ligation products over the expected total coverage of captured products, with the latter being estimated based on a probabilistic background model. Local changes in color codes indicate regions 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 (0.6kb) containing the major allele for rs6781009, which is reduced for the minor allele in both a large enhancer construct (1.5kb), as well as in the core enhancer element (0.6kb) **P*<0.01 (**d**) Dorsal views of hearts containing the human regulatory element with the major vs 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. ra, right atrium; la, left atrium; rv, right ventricle; lv, left ventricle.

Fig. 4 Cardiac defects upon heart-specific RNAi knockdown in *Drosophila*. (**a**) Wild-type dorsal heart tube stained with the F-actin stain phalloidin. Magnified region (right) is highlighted. Arrowheads point to ostia (inflow tracks), 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), while some heart-associated longitudinal muscles are still present (arrowheads). At larval stages the heart is much less affected, suggesting maturation or remodeling defect. (**d**) Knockdown of EcR/NR1H blocks cardiac remodeling and causes myofibrillar disarray (arrow). Ventral longitudinal muscles are also abnormal (arrowhead).

Fig. 5 DEPICT analysis. (**a**) Plots showing the enrichment of loci associated with QRS traits in specific physiological systems. (**b**) Graphical display of DEPICT gene set enrichment analysis. Gene meta-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).

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