

# Identification of heart rate–associated loci and their effects on cardiac conduction and rhythm disorders

Elevated resting heart rate is associated with greater risk of cardiovascular disease and mortality. In a 2-stage meta-analysis of genome-wide association studies in up to 181,171 individuals, we identified 14 new loci associated with heart rate and confirmed associations with all 7 previously established loci. Experimental downregulation of gene expression in *Drosophila melanogaster* and *Danio rerio* identified 20 genes at 11 loci that are relevant for heart rate regulation and highlight a role for genes involved in signal transmission, embryonic cardiac development and the pathophysiology of dilated cardiomyopathy, congenital heart failure and/or sudden cardiac death. In addition, genetic susceptibility to increased heart rate is associated with altered cardiac conduction and reduced risk of sick sinus syndrome, and both heart rate–increasing and heart rate–decreasing variants associate with risk of atrial fibrillation. Our findings provide fresh insights into the mechanisms regulating heart rate and identify new therapeutic targets.

A high resting heart rate has been associated with increased incidence of cardiovascular disease, as well as with cardiovascular and all-cause mortality, independent of traditional risk factors<sup>1–3</sup>. There are several potential mechanisms by which higher heart rate may contribute to greater cardiovascular risk. For example, higher heart rate entails elevated myocardial oxygen requirement and a shift in cardiac control from parasympathetic to sympathetic dominance, which may increase the likelihood of myocardial ischemia and electrical instability<sup>4</sup>. In addition, experimental alteration of heart rate by sinoatrial node ablation has been shown to influence the progression of atherosclerosis induced by an atherogenic high-cholesterol diet in cynomolgus monkeys<sup>5,6</sup>. In humans, selective reduction of heart rate using ivabradine was shown to reduce clinical events in individuals with heart failure, suggesting that elevated heart rate is a clinically relevant and modifiable risk factor<sup>7</sup>. However, whether the association of higher heart rate with cardiovascular risk is causal remains to be clarified.

Large twin studies with electrocardiogram (ECG) data have shown that genetic factors contribute to interindividual variation in heart rate, with heritability estimates ranging from 55 to 77% (refs. 8–10). So far, 3 genome-wide association studies (GWAS)<sup>11–13</sup>, incorporating data from up to 38,991 individuals each, have identified variants in 7 loci that show evidence of association with heart rate. These variants are common in the general population (minor allele frequency (MAF)  $\geq 10\%$ ) and together explain  $\sim 0.7\%$  of the variance in heart rate<sup>12</sup>. To gain more comprehensive insight into the genetic regulation of heart rate, we performed a 2-stage meta-analysis of GWAS in data from up to 181,171 individuals. Loci convincingly associated with heart rate were subsequently tested for association with cardiac conduction, rhythm disorders and cardiovascular disease to elucidate potential mechanisms underlying the association between heart rate and cardiovascular disease and mortality. Furthermore, we undertook experimental studies in *D. melanogaster* and *D. rerio* models as a first step toward identifying the causal genes within the associated loci.

## RESULTS

### Stage 1 GWAS identifies five new loci associated with heart rate

We performed a meta-analysis of the associations between 2,516,789 SNPs and heart rate in data from up to 85,787 individuals of European ancestry from 36 GWAS, including data from up to 11,207 individuals described previously<sup>13</sup> and 6,568 individuals of Indian Asian ancestry (Online Methods, **Supplementary Figs. 1–3** and **Supplementary Tables 1–4**). All studies included have been approved by local ethics committees, and all participants have provided their consent in writing. Our stage 1 meta-analysis showed associations with heart rate at genome-wide significance ( $P < 5 \times 10^{-8}$ ) for variants in 12 loci (**Table 1**). These 12 loci included all 7 previously identified loci (in *MYH6*, *CD46* and *FADS1* and near *GJA1*, *ACHE*, *SLC35F1* and *LINC00477* (also known as *CI2orf67*))<sup>11–13</sup> and 5 additional loci (in *KIAA1755*, *CCDC141*, *SYT10* and *FLRT2* and near *HCN4*).

To validate associations of the loci that were significantly associated with heart rate in stage 1 and to identify additional loci (**Supplementary Fig. 1**), lead SNPs at 42 loci (associated at  $P < 3 \times 10^{-5}$ ) were selected for follow-up (Online Methods and **Supplementary Table 5**). Conditional analyses based on summary statistics of stage 1 meta-analysis results<sup>14</sup> identified two loci with secondary associations that remained significant ( $P < 5 \times 10^{-8}$ ) after adjusting for the association of the lead SNP. These secondary associations were also selected for follow-up (Online Methods and **Supplementary Table 6**).

### Stage 2 follow-up identifies nine additional new loci

In stage 2, we examined associations between the 42 loci identified in stage 1 and heart rate in data from up to 88,823 additional individuals of European descent from 27 GWAS, including data from up to 38,991 individuals from 15 GWAS described previously<sup>12</sup>, as well as 11 studies with Metachip and 1 study with Cardiochip data (Online Methods, **Supplementary Fig. 1** and **Supplementary Tables 7–10**).

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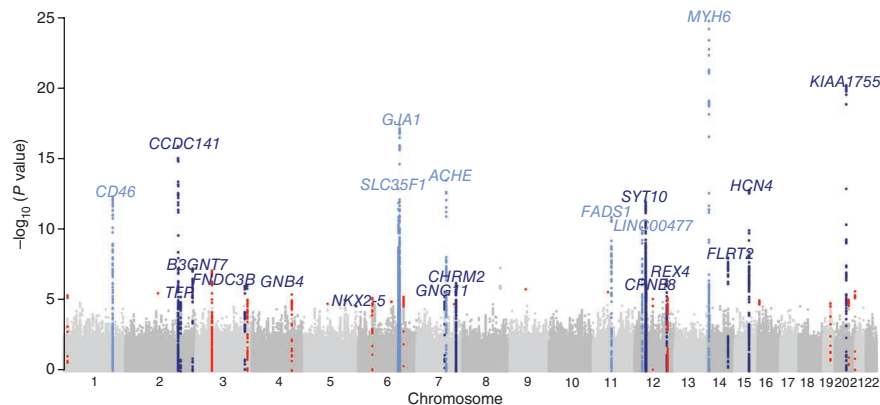

**Table 1 Stage 1 and stage 2 results for the loci that showed association with heart rate at genome-wide significance ( $P < 5 \times 10^{-8}$ )**

Locus	Chr.	Nearest gene	Annotation	Heart rate SNP	Position (bp) <sup>a</sup>	Alleles		Per-allele change in heart rate <sup>b</sup>			Stage 1		Stage 2		Stages 1 + 2	
						Effect	Other	EAF	$\beta$	SE	$P$	$P$	$N$	$P$	$N$	
<b>Previously identified heart rate loci</b>																
1	14	<i>MYH6</i>	N,P,S	rs365990	22931651	G	A	0.353	0.564	0.047	$5.66 \times 10^{-28}$	$2.19 \times 10^{-21}$	148,972	$5.39 \times 10^{-45}$		
2	6	<i>GJA1</i>	S	rs1015451	122173184	C	T	0.102	0.713	0.072	$7.06 \times 10^{-20}$	$2.14 \times 10^{-17}$	155,303	$1.14 \times 10^{-33}$		
3	7	<i>ACHE</i>	N,Q,S,B	rs13245899	100335067	G	A	0.195	0.447	0.055	$1.54 \times 10^{-15}$	$3.27 \times 10^{-14}$	176,643	$7.67 \times 10^{-27}$		
4	1	<i>CD46</i>	S,B	rs11118555	206007476	A	T	0.124	0.612	0.069	$4.35 \times 10^{-14}$	$7.49 \times 10^{-15}$	166,654	$3.88 \times 10^{-26}$		
5	11	<i>FADS1</i>	Q,S,B	rs174549	61327958	A	G	0.310	0.358	0.047	$1.13 \times 10^{-12}$	$1.57 \times 10^{-12}$	172,847	$1.38 \times 10^{-22}$		
6	6	<i>SLC35F1</i>	T,Q,P,S	rs11153730	118774215	T	C	0.509	0.381	0.044	$7.01 \times 10^{-15}$	$8.79 \times 10^{-9}$	156,783	$7.55 \times 10^{-21}$		
7	12	<i>LINC00477 (C12orf67)</i>		rs17287293	24662145	A	G	0.850	0.444	0.062	$6.90 \times 10^{-12}$	$3.98 \times 10^{-11}$	151,085	$3.07 \times 10^{-20}$		
<b>Newly identified heart rate loci</b>																
8	20	<i>KIAA1755</i>	N,S	rs6127471	36277452	C	T	0.540	0.429	0.045	$5.96 \times 10^{-23}$	$2.98 \times 10^{-10}$	162,593	$5.22 \times 10^{-29}$		
9	2	<i>CCDC141</i>	N,P,S	rs17362588	179429291	A	G	0.114	0.736	0.077	$3.25 \times 10^{-18}$	$4.22 \times 10^{-11}$	136,061	$3.57 \times 10^{-26}$		
10	12	<i>SYT10</i>		rs7980799	33468257	A	C	0.401	0.377	0.046	$7.91 \times 10^{-14}$	$7.87 \times 10^{-13}$	166,043	$6.22 \times 10^{-24}$		
11	15	<i>HCN4</i>	S	rs4489968	71452559	T	G	0.843	0.513	0.060	$8.89 \times 10^{-15}$	$3.34 \times 10^{-8}$	160,858	$3.82 \times 10^{-20}$		
12	3	<i>GMB4</i>	S	rs7612445	180655673	G	T	0.816	0.358	0.060	$2.41 \times 10^{-7}$	$7.78 \times 10^{-10}$	140,395	$1.86 \times 10^{-14}$		
13	14	<i>FLRT2</i>	S	rs17796783	84879664	T	C	0.716	0.334	0.049	$1.65 \times 10^{-9}$	$5.76 \times 10^{-6}$	145,835	$2.69 \times 10^{-13}$		
14	7	<i>CHRM2</i>	S	rs2350782	136293174	C	T	0.116	0.505	0.078	$1.57 \times 10^{-7}$	$2.63 \times 10^{-7}$	131,781	$1.26 \times 10^{-12}$		
15	5	<i>NKX2-5</i>	Q,S	rs6882776	172596769	G	A	0.680	0.301	0.051	$9.67 \times 10^{-6}$	$7.43 \times 10^{-9}$	158,807	$2.29 \times 10^{-12}$		
16	7	<i>GNG11</i>	C,Q,S,B	rs180242	93387532	T	A	0.333	0.316	0.053	$7.52 \times 10^{-7}$	$5.59 \times 10^{-7}$	148,111	$6.78 \times 10^{-12}$		
17	2	<i>B3GNT7</i>	N,Q,S,P,B	rs13030174	231979528	A	C	0.733	0.300	0.051	$1.13 \times 10^{-8}$	$3.66 \times 10^{-4}$	144,810	$1.04 \times 10^{-10}$		
18	3	<i>FNDCC3B</i>	S	rs9647379	173267862	C	G	0.400	0.206	0.047	$2.59 \times 10^{-7}$	$3.07 \times 10^{-4}$	137,314	$1.17 \times 10^{-9}$		
19	12	<i>RFK4</i>	S	rs2067615	105673552	A	T	0.490	0.278	0.044	$1.05 \times 10^{-7}$	$8.49 \times 10^{-4}$	151,197	$1.58 \times 10^{-9}$		
20	12	<i>CPNE8</i>		rs2626838	37392998	C	T	0.443	0.234	0.045	$3.32 \times 10^{-7}$	$6.20 \times 10^{-4}$	166,632	$3.73 \times 10^{-9}$		
21	2	<i>TFPI</i>	N,S	rs4140885	188041309	A	G	0.317	0.217	0.049	$1.85 \times 10^{-6}$	$1.40 \times 10^{-3}$	170,395	$4.72 \times 10^{-8}$		

Chr., chromosome; EAF, effect allele frequency based on meta-analysis of stages 1 and 2 combined. Annotation shows whether the heart rate-associated SNP is (i) in strong LD ( $r^2 > 0.8$ ) with a copy number variant (CN), nonsynonymous variant (N) or variant in a transcription factor binding site (TF); (ii) associated with an eQTL; (Q); (iii) expressed at the protein level in mouse heart and phosphorylated upon stimulation of the  $\beta$ 1AR (P); (iv) located in or near a gene that was identified as being of potential relevance for heart rate using the automated literature search program SNIPPER (S); or (v) located in or near a biological candidate gene for heart rate (B).

<sup>a</sup>Positions are according to HapMap Build 36, and allele coding is based on the positive strand. <sup>b</sup>Effect sizes in bpm per effect allele obtained from stage 1 and stage 2 cohorts with heart rate as only outcome ( $N > 96,790$ ; excludes data from the RRGen Consortium and ERF).

**Figure 1** Manhattan plot of SNPs after meta-analysis of stage 1. The plot shows the significance of associations between all SNPs and heart rate in stage 1. The 7 loci that were previously identified are highlighted in light blue; the 14 newly associated loci are highlighted in dark blue. Loci that reached  $P < 3 \times 10^{-5}$  after stage 1 but did not reach  $P < 5 \times 10^{-8}$  after meta-analysis of stages 1 and 2 combined are highlighted in red.



In a joint analysis of results from stage 1 and stage 2, variants in 21 loci had associations that reached  $P < 5 \times 10^{-8}$  in data from up to 181,171 individuals (**Fig. 1**, **Table 1** and **Supplementary Table 5**). Among the 21 loci were all 12 loci with association  $P < 5 \times 10^{-8}$  after stage 1, as well as 9 additional loci (in *CHRM2*, *RFX4*, *CPNE8* and *TFPI* and near *GNB4*, *NKX2-5*, *GNG11*, *B3GNT7* and *FNDC3B*). Hence, our study confirms the 7 previously identified loci<sup>11–13</sup> and identifies 14 new loci robustly associated with heart rate.

### Impact of the 21 confirmed loci on heart rate

The frequency of the heart rate-increasing alleles ranged from 10 to 85% for the 21 confirmed associations. Effect sizes of associations ranged from 0.21 to 0.74 beats per minute (bpm) per effect allele (mean  $\pm$  s.d.,  $0.41 \pm 0.15$  bpm per effect allele) (**Fig. 2** and **Table 1**).

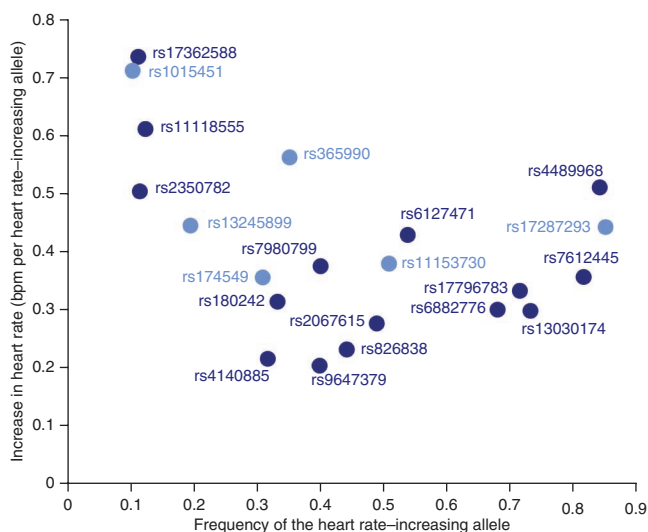
To estimate the combined effect of the 21 loci on heart rate, we constructed a genetic predisposition score (GPS) by summing the number of heart rate-increasing alleles of the 21 associations. We examined associations between the GPS and heart rate in data from 5,053 adults from LifeLines2 (data for 19 loci available) and 4,000 12-year-old children from ALSPAC (data for 21 loci available) (Online Methods). The difference in average heart rate between individuals in the lowest and highest 5% of the GPS distribution was 4.1 bpm in adults (66.1 versus 70.2 bpm) and 4.9 bpm in children (73.7 versus 78.6 bpm) (**Fig. 3a,b**), differences that were previously shown to be clinically relevant<sup>15</sup>. The GPS explained 0.9% of the variance in heart rate in adults from LifeLines2 and 0.8% of the variance in children from ALSPAC.

### Conduction, rhythm disorders and cardiovascular disease

An altered heart rate reflects sinoatrial function and may reflect disturbed electrophysiological properties that are also present in other

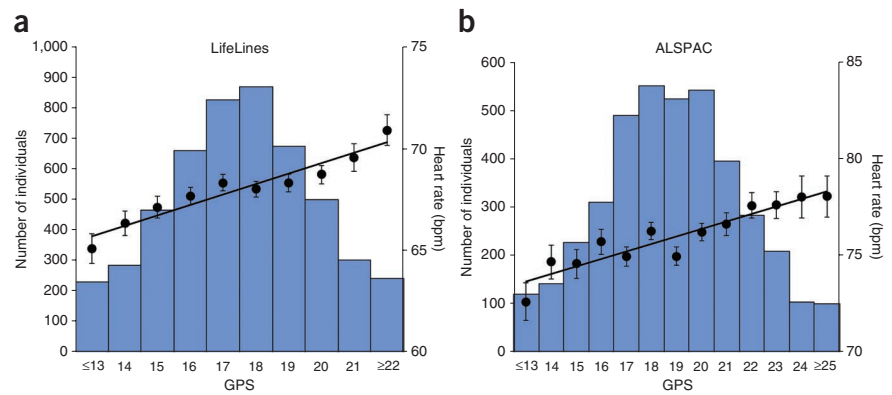
compartments of the heart. Such properties include atrial and atrioventricular nodal conduction (PR duration), ventricular depolarization (QRS duration) and myocardial repolarization (QT duration), which can be quantified on a 12-lead ECG. We examined whether the heart rate-associated loci showed evidence of association with cardiac conduction in data from previously reported GWAS for PR<sup>16</sup>, QRS<sup>17</sup> and QT duration (QT-IGC Consortium (C.N.-C.), personal communication). Furthermore, we examined the association of the 21 loci with the risk of several conduction-related disorders, including atrial fibrillation, advanced (second- and third-degree) atrioventricular block and sick sinus syndrome (SSS, also known as sinus node dysfunction), as well as pacemaker implantation and sudden cardiac death<sup>13,18</sup>. Finally, elevated resting heart rate is a well-recognized precursor of increased blood pressure and hypertension, independent of initial blood pressure levels<sup>19,20</sup>, and predicts the incidence of coronary heart disease during up to 10 years of follow-up, independent of other major risk factors<sup>1,2</sup>. We therefore also examined associations of the heart rate loci with systolic blood pressure, diastolic blood pressure and the prevalence of hypertension, coronary artery disease (CAD) and myocardial infarction in data from the Global BPgen<sup>21</sup> and CARDIoGRAM consortia<sup>22</sup> (Online Methods).

For each of the ECG traits, we found a significant association with individual heart rate loci ( $P < 0.002$ ). Heart rate-increasing alleles of these loci were associated with prolonged PR duration (near *LINC00477* and *NKX2-5*) and reduced QT duration (near *GJA1*, *FADS1*, *SLC35F1* and *NKX2-5*), independent of heart rate, as well as with both reduced (near *GJA1*, *FADS1*, *SLC35F1* and *NKX2-5*) and prolonged (in *CCDC141*) QRS duration (**Table 2**). Common variants of the loci in or near *GJA1*, *FADS1*, *CCDC141* and *NKX2-5* were not previously identified as being associated with these cardiac conduction traits (**Supplementary Table 11**). In addition, stronger genetic susceptibility for increased heart rate as conferred by the GPS of 21 loci was associated with prolonged PR duration ( $P = 1.3 \times 10^{-4}$ ) and reduced QT duration ( $P = 1.1 \times 10^{-17}$ ), independent of heart rate, as well as with reduced QRS duration ( $P = 1.8 \times 10^{-5}$ ) (**Table 2** and **Supplementary Fig. 4**). These results suggest that, to some extent, similar cellular processes control heart rate and cardiac conduction through the atria and ventricles.



**Figure 2** Effect size as a function of effect allele frequency. The plot shows the effect sizes of the 21 heart rate-associated SNPs after joint meta-analysis of stage 1 and stage 2 results as a function of their effect allele frequencies. Light-blue circles represent the 7 previously identified heart rate loci; dark-blue circles represent the 14 newly identified heart rate loci.

**Figure 3** Combined effect of heart rate-increasing alleles on heart rate. (a) Combined effect of the 19 available heart rate loci in adults of European descent (LifeLines2,  $n = 5,053$ ). (b) Combined effect of the 21 heart rate loci in 12-year-old children of European descent (ALSPAC,  $n = 4,000$ ). In each plot, the number of heart rate-increasing alleles was summed across the heart rate-associated SNPs. The number of heart rate-increasing alleles is shown ( $x$  axis), grouped at the extremes, and mean heart rate  $\pm$  s.e.m. is plotted (right  $y$  axis). The lines represent the regression of the mean heart rate values across the GPS distribution. The histogram shows the number of individuals in each GPS window (left  $y$  axis).



Five of the 21 heart rate loci are associated with atrial fibrillation ( $P < 0.002$ ). Heart rate-increasing alleles of these loci were associated with both increased (near *SLC35F1*, *LINC00477* and *NKX2-5*; odds ratio (OR) = 1.06–1.13) and decreased (near *GJA1* and *HCN4*; OR = 0.86–0.90) risk of atrial fibrillation (Table 2). Common variants of the loci in or near *GJA1*, *SLC35F1* and *NKX2-5* were not previously identified as being associated with atrial fibrillation (Supplementary Table 11). Stronger genetic susceptibility for increased heart rate in the 21 loci combined was not associated with atrial fibrillation, which reflects the bidirectionality of the associations in the individual loci (Table 2).

None of the heart rate loci showed evidence of association with the risk of atrioventricular block, SSS, pacemaker implantation or sudden cardiac death individually (Supplementary Table 12). However, a higher GPS was associated with reduced risk of SSS ( $P = 2.3 \times 10^{-4}$ ) and pacemaker implantation ( $P = 3.6 \times 10^{-4}$ ) (Table 2 and Supplementary Fig. 4). SSS encompasses a group of sinus rhythm disorders, including pathological sinus bradycardia (slow heart rate), sinus arrest, sinoatrial block and paroxysmal tachycardias (bradycardia-tachycardia syndrome). SSS is the most common indicator for permanent pacemaker implantation<sup>23</sup>, and ~80% of individuals with SSS in our data set had undergone pacemaker implantation<sup>18</sup>. Hence, the association between the heart rate loci and pacemaker implantation is likely secondary to the association with SSS in this study population.

None of the heart rate loci showed evidence of association with blood pressure or prevalent hypertension, CAD or myocardial infarction, either individually or when combined in the GPS of 21 loci (Supplementary Fig. 4 and Supplementary Tables 13 and 14). In addition, we showed, at most, limited evidence of association with heart rate for loci previously identified as being associated with blood pressure or prevalent hypertension, CAD or myocardial infarction (Supplementary Tables 15 and 16).

### Pathway analyses

The 21 confirmed loci contain 234 genes that are located within 500 kb of the associations with heart rate (Supplementary Fig. 5 and Supplementary Table 17). To systematically identify biological connections between these genes and to identify new pathways associated with heart rate, we tested whether biological processes or molecular functions that were predefined in five databases were enriched for multiple modest heart rate associations using MAGENTA<sup>24</sup> (Online Methods). We found evidence of enrichment of associations in pathways involved in dilated, hypertrophic and arrhythmogenic right ventricular cardiomyopathy, (cardiac) muscle contraction, regulation of

heart contraction, integration of energy metabolism, positive regulation of cell adhesion and Alzheimer's disease ( $P < 2 \times 10^{-3}$ , false discovery rate (FDR) < 0.1) (Supplementary Tables 18 and 19).

### Follow-up in *D. melanogaster* and *D. rerio*

Variants identified by GWAS typically implicate genomic regions rather than individual genes. We used a range of approaches to identify promising candidate genes for heart rate regulation within the 21 loci, including proteomics experiments aimed at identifying genes expressed at the protein level in mouse heart that are phosphorylated upon stimulation of the  $\beta 1$  adrenergic receptor ( $\beta 1$ AR), gene expression quantitative trait locus (eQTL) analysis in blood, *in silico* search for potentially functional variants in high linkage disequilibrium (LD) with lead variants ( $r^2 > 0.8$ ), an automated literature search using the program SNIPPER and biological candidacy (Online Methods and Supplementary Tables 20–23). These approaches labeled 49 of the 234 genes located within the 21 loci as candidate genes for heart rate regulation (Supplementary Table 24).

To examine whether some of the 49 candidate genes are likely to underlie the associations identified by GWAS, we performed 2 series of experiments using animal models. First, we compared heart rate and risk of arrhythmia in *D. melanogaster* control pupae and pupae in which orthologs of the candidate genes were downregulated using RNA-mediated gene interference (RNAi), both at rest and after 20 min of tachypacing (Online Methods and Supplementary Tables 25 and 26). Second, we compared heart rate and fractional shortening of the ventricular chamber in control embryos of the zebrafish *D. rerio* and embryos in which orthologs of the candidate genes were downregulated using morpholino oligonucleotides (Online Methods and Supplementary Table 27).

Results were available for the orthologs of 25 candidate genes from 13 loci in *D. melanogaster* pupae and for orthologs of 12 genes from 7 loci in *D. rerio* embryos; results from orthologs of 6 genes were available in both species (Supplementary Fig. 6 and Supplementary Table 24). Results from these experiments support a role in heart rate regulation for 20 of the 31 candidate genes tested across the 2 models: *ACHE*, *UFSP1*, *TRIP6*, *EPHB4* and *PCOLCE* (locus 3), *PLXNA2* (locus 4), *FADS1*, *FADS2*, *FADS3* and *BEST1* (locus 5), *TTN* (locus 9), *MFN1* (locus 12), *CHRM2* (locus 14), *GNG11* (locus 15), *NCL* and *HTR2B* (locus 17), *PLD1* and *GHSR* (locus 18), *CRY1* (locus 19) and *CALCRL* (locus 21) ( $P < 2 \times 10^{-3}$  in *D. melanogaster* pupae and  $P < 4 \times 10^{-3}$  in *D. rerio* embryos) (Fig. 4 and Supplementary Tables 28 and 29).

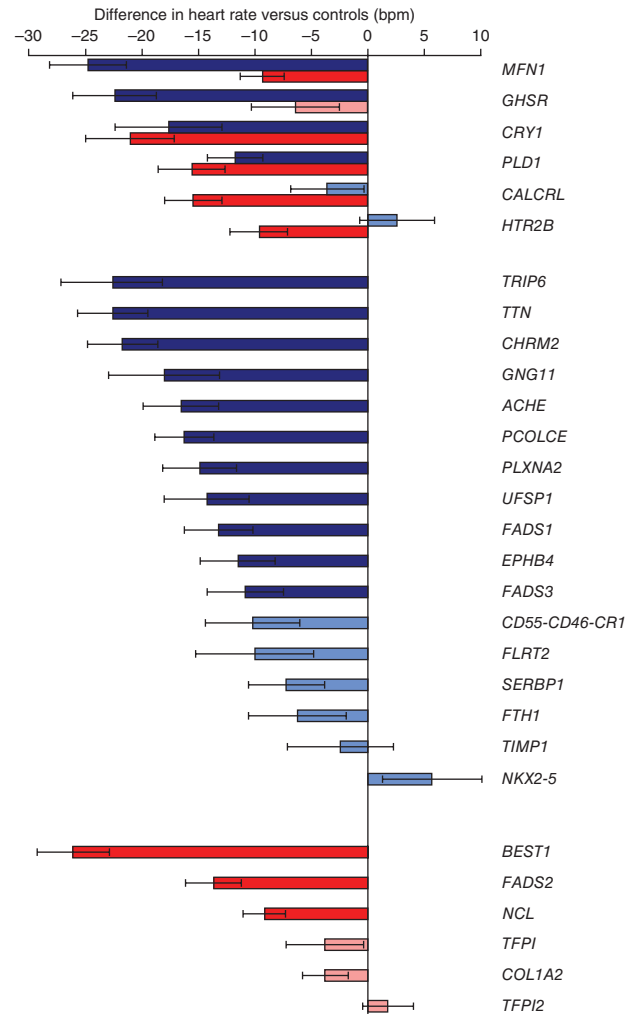
The most convincing results were observed for orthologs of *MFN1* and *PLD1* (Supplementary Note), for which downregulated gene expression reduced resting heart rate in both models ( $P < 1 \times 10^{-5}$ ).

Table 2 Association of the heart rate loci with cardiac conduction and rhythm disorders

Locus	Nearest gene	Heart rate SNP	Alleles			PR duration			QRS duration			QT duration			Atrial fibrillation			SSS												
			Effect	Other	N	$\beta$ (ms)	SE (ms)	P	N	$\beta$ (ms)	SE (ms)	P	N	$\beta$ (ms)	SE (ms)	P	OR (95% CI)	P	OR (95% CI)	P										
<b>Previously identified heart rate loci</b>																														
1	<i>MYH6</i>	rs365990	G	A	22,711	-0.53	0.23	0.02	36,680	-0.00	0.07	0.98	67,414	0.05	0.10	0.65	1.027	0.12	0.931	0.18										
2	<i>GJA1</i>	rs1015451	C	T	26,528	0.66	0.34	0.05	40,090	-0.38	0.11	$6.51 \times 10^{-4}$	72,806	-0.50	0.16	$1.67 \times 10^{-3}$	0.903	$1.58 \times 10^{-4}$	0.961	0.63										
3	<i>ACHE</i>	rs13245899	G	A	25,317	0.35	0.27	0.19	37,902	-0.07	0.09	0.41	72,980	0.11	0.12	0.39	0.985	0.46	0.951	0.46										
4	<i>CD46</i>	rs11118555	A	T	27,701	-0.03	0.32	0.93	39,530	-0.00	0.11	0.97	73,601	-0.25	0.15	0.09	0.964	0.15	0.895	0.16										
5	<i>FADS1</i>	rs174549	A	G	28,482	-0.53	0.22	0.02	40,085	-0.27	0.07	$1.85 \times 10^{-4}$	74,604	-0.57	0.10	$4.33 \times 10^{-8}$	1.003	0.85	0.971	0.57										
6	<i>SLC35F1</i>	rs11153730	T	C	27,585	0.56	0.20	$6.20 \times 10^{-3}$	39,200	-0.59	0.07	$1.26 \times 10^{-18}$	74,932	-1.65	0.10	$2.23 \times 10^{-67}$	1.061	$5.19 \times 10^{-4}$	0.973	0.59										
7	<i>LINC00477</i> ( <i>C12orf67</i> )	rs17287293	A	G	27,912	2.06	0.29	$4.91 \times 10^{-13}$	39,548	-0.10	0.09	0.31	74,180	-0.01	0.14	0.93	1.132	$2.38 \times 10^{-7}$	0.811	$2.83 \times 10^{-3}$										
<b>Newly identified heart rate loci</b>																														
8	<i>KIAA1755</i>	rs6127471	C	T	27,459	0.47	0.21	0.02	39,775	-0.06	0.07	0.40	72,570	-0.28	0.10	$4.17 \times 10^{-3}$	0.978	0.18	0.973	0.61										
9	<i>CCDC141</i>	rs17362588	A	G	24,111	-0.83	0.33	0.01	35,425	0.55	0.11	$4.75 \times 10^{-7}$	64,780	0.27	0.16	0.09	1.016	0.55	0.873	0.09										
10	<i>SYT10</i>	rs7980799	A	C	24,965	0.52	0.22	0.02	36,338	0.03	0.07	0.64	69,431	-0.18	0.10	0.08	0.990	0.57	1.027	0.60										
11	<i>HCN4</i>	rs4489968	T	G	25,991	0.67	0.29	0.02	38,579	0.09	0.09	0.33	72,387	0.05	0.13	0.69	0.856	$1.85 \times 10^{-12}$	0.924	0.26										
12	<i>GNB4</i>	rs7612445	G	T	23,153	-0.37	0.28	0.18	38,398	-0.14	0.09	0.11	67,376	-0.11	0.13	0.40	0.959	0.04	0.912	0.14										
13	<i>FLRT2</i>	rs17796783	T	C	27,993	-0.12	0.22	0.61	38,467	-0.05	0.07	0.54	73,380	-0.24	0.11	0.02	0.959	0.02	0.898	0.05										
14	<i>CHRM2</i>	rs2350782	C	T	23,414	-0.26	0.37	0.48	34,417	-0.07	0.12	0.57	58,549	0.25	0.19	0.17	0.941	0.05	0.911	0.32										
15	<i>MKX2-5</i>	rs6882776	G	A	18,484	1.29	0.27	$1.46 \times 10^{-6}$	30,877	-0.27	0.09	$1.87 \times 10^{-3}$	60,768	-0.49	0.12	$2.27 \times 10^{-5}$	1.110	$4.41 \times 10^{-8}$	1.056	0.33										
16	<i>GNG11</i>	rs180242	T	A	21,101	0.33	0.24	0.17	34,141	0.00	0.08	0.96	63,609	0.02	0.11	0.84	0.995	0.80	1.025	0.63										
17	<i>B3GN7</i>	rs13030174	A	C	27,169	0.18	0.24	0.45	38,446	-0.23	0.08	$4.14 \times 10^{-3}$	72,111	-0.14	0.11	0.19	0.997	0.88	1.025	0.67										
18	<i>FND3B</i>	rs9647379	C	G	21,503	0.65	0.23	$4.23 \times 10^{-3}$	35,642	-0.02	0.07	0.74	63,885	0.03	0.10	0.74	1.002	0.89	0.969	0.55										
19	<i>RFY4</i>	rs2067615	A	T	27,782	-0.17	0.20	0.40	39,494	-0.05	0.07	0.42	74,116	-0.02	0.10	0.83	1.029	0.08	1.054	0.29										
20	<i>CPNE8</i>	rs826838	C	T	28,059	0.62	0.21	$2.78 \times 10^{-3}$	39,313	0.02	0.07	0.80	74,402	-0.04	0.10	0.71	0.999	0.93	0.926	0.12										
21	<i>TFF1</i>	rs4140885	A	G	26,384	-0.15	0.23	0.50	37,447	0.05	0.08	0.55	68,919	-0.18	0.11	0.10	1.011	0.55	1.126	0.02										
Multi-SNP predisposition score												2.55	0.67	$1.33 \times 10^{-4}$	-0.95	0.22	$1.83 \times 10^{-5}$	-2.66	0.31	$1.12 \times 10^{-17}$	0.921	0.11	0.589	$2.34 \times 10^{-4}$						

Association analyses were performed in data from the PR GWAS Consortium (PR duration), the QRS GWAS Consortium (QRS duration), the QT-IGC Consortium (QT duration), deCODE Genetics and the CHARGE-AF Consortium (atrial fibrillation), and deCODE Genetics (SSS). Analyses were performed in data from 9,183 cases and 91,625 controls for atrial fibrillation and in data from 903 cases and 40,722 controls for SSS. CI, confidence interval; effect allele, the allele that is associated with higher heart rate. P values for association with PR duration are not adjusted for population stratification, as are the ones for the remaining traits. The multi-SNP predisposition score shows the combined effect of the  $Z_1$  heart rate-associated SNPs per genetically predicted 5-bpm increase in heart rate, based on single-SNP summary statistics and weighting by effect sizes for heart rate. P values smaller than  $2.0 \times 10^{-3}$  are considered statistically significant.

**Figure 4** Effects on heart rate of reduced or ablated expression of orthologs of positional candidate genes from GWAS in *D. melanogaster* and *D. rerio*. Bars show the heart rate ( $\pm$  s.e.m.) of (i) *D. melanogaster* pupae with orthologs of positional candidate genes located within 500 kb of GWAS associations downregulated using RNAi compared with control pupae (blue bars) and (ii) *D. rerio* (zebrafish) embryos with expression of orthologs of positional candidate genes reduced by injecting morpholino oligonucleotides versus PBS (red bars). Darker coloring indicates that heart rate is significantly different in targeted animals compared with controls after Bonferroni correction for 23 tests in *D. melanogaster* ( $P < 2 \times 10^{-3}$ ) and 12 tests in *D. rerio* ( $P < 4 \times 10^{-3}$ ); lighter coloring indicates that differences do not reach significance. Results are ordered by availability (*D. melanogaster* and *D. rerio*, *D. melanogaster* only, *D. rerio* only) and by effect size.



Moreover, *D. melanogaster* pupae with downregulated expression of the *MFN1* ortholog were characterized by reduced heart rate after 20 min of tachypacing ( $P = 9.5 \times 10^{-5}$ ) and by increased risk of arrhythmia, both at rest ( $P = 4.3 \times 10^{-4}$ ) and after tachypacing ( $P = 2.0 \times 10^{-6}$ ). In *D. rerio* embryos, in addition to reduced heart rate, downregulation of *mfn1* (the ortholog of *MFN1*) was accompanied by edema in 73% of embryos, whereas 51% of embryos with reduced expression of *pld1a* (the ortholog of *PLD1*) had an unlooped heart (Supplementary Table 29).

In addition to reduced heart rate, downregulated gene expression of orthologs of *ACHE*, *PCOLCE* and *FADS3* was associated with increased risk of arrhythmia after 20 min of tachypacing in *D. melanogaster* pupae. Furthermore, *D. rerio* embryos with reduced gene expression of *D. rerio* orthologs that was accompanied by reduced heart rate were also characterized by edema (*BEST1* and *MFN1*), blood pooling (*FADS2*), an unlooped heart (*HTR2B*, *NCL*, *PLD1* and *CALCRL*) and atrioventricular canal malformation (*CALCRL*). Downregulated expression of the *CRY1* ortholog was accompanied by a highly penetrant developmental malformation that likely mediates the heart rate effect (Supplementary Fig. 7 and Supplementary Table 29). Reduced expression of the *COL1A2* ortholog did not affect heart rate in *D. rerio* embryos ( $P = 0.07$ ) but resulted in reduced fractional shortening of the ventricular chamber ( $P = 1.8 \times 10^{-3}$ ).

In summary, results from experiments in *D. melanogaster* and *D. rerio* models support a role in heart rate regulation for 20 genes found within 11 loci associated with heart rate (Supplementary Table 30). Notably, results from animal models confirmed the eQTL associations observed in humans for all available orthologs, with a consistent direction of effect across species for all genes except *TRIP6* (Supplementary Tables 21 and 30).

## DISCUSSION

Using a 2-stage meta-analysis of GWAS in up to 181,171 individuals, we identified 14 loci previously unknown to be robustly associated with heart rate and confirmed the 7 previously established loci, increasing the total number of heart rate loci to 21. Results from experiments in *D. melanogaster* and *D. rerio* models support a role in heart rate regulation for 20 candidate genes from 11 loci. These experiments highlight a role for genes that are essential for embryonic cardiovascular development and signal transmission, as well as for genes with a role in the pathophysiology of dilated cardiomyopathy, congestive heart failure and/or sudden cardiac death (Supplementary Note). In addition, stronger genetic susceptibility to higher heart rate is associated with prolonged PR duration and reduced QT duration, both independent of heart rate, as well as with reduced QRS duration

and lower risk of SSS, a group of sinus rhythm disorders that result from sinus node dysfunction and are characterized by bradycardia.

Prevalent SSS is unlikely to explain the association between common variants and heart rate shown by GWAS, as the associations were essentially unchanged with a priori exclusion of individuals with prevalent cardiovascular disease, heart rate outside the range of 50–100 bpm and/or using heart rate-altering medication. This suggests that the confirmed loci have subtle effects on sinus node function in the general population, which manifest themselves in higher heart rate and reduced risk of SSS, showing the clinical relevance of our findings. Future studies should address whether such effects on sinus node function also affect the risk of mortality. The associations with higher heart rate do not translate into significantly higher risk of CAD or myocardial infarction, either individually or in combination, which may reflect low statistical power given the effect sizes for heart rate and the number of available CAD and myocardial infarction cases.

Heart rate-increasing alleles of the loci near *GJA1*, *SLC35F1* (*PLN*) and *NKX2-5* show unidirectional associations with reduced QRS and QT duration but bidirectional associations with atrial fibrillation. These findings suggest that both tails of the ventricular depolarization and myocardial repolarization distributions are associated with increased risk of atrial fibrillation and that altered heart rate associated with genetic predisposition in these loci may reflect adaptations to disturbed electrophysiological properties (compartments) of the heart. Results from experiments in a *D. melanogaster* model highlight

genes in additional loci that show suggestive evidence of a role in both heart rate regulation and arrhythmia susceptibility (*ACHE*, *PCOLCE*, *FADS3* and *MFN1*). Taken together, these results may enable the discovery of new druggable targets for the prevention and treatment of cardiovascular endpoints by selective reduction of heart rate and arrhythmia susceptibility, similar to the way ivabradine likely exerts its effects through targeting of *HCN4* (refs. 7,25).

Results from experiments in *D. melanogaster* and *D. rerio* models support a role in heart rate regulation for genes that are essential for embryonic cardiovascular development (*EPHB4*, *PLXNA2*, *PLD1* and *CALCRL*), as well as for genes with a role in the pathophysiology of dilated cardiomyopathy, congestive heart failure and/or sudden cardiac death (*TTN*, *MFN1*, *CHRM2* and *PLD1*). In congruence, we show that zebrafish embryos with downregulated expression of orthologs of these genes have edema (*MFN1*), an unlooped heart (*PLD1* and *CALCRL*) and atrioventricular canal malformation (*CALCRL*). Such defects in cardiovascular development can be hypothesized to mediate the reduced heart rate that we observe in these embryos. Future studies are required to determine whether individuals with genetic susceptibility for reduced heart rate in these loci are enriched for mild forms of such cardiovascular phenotypes.

In conclusion, our results provide new insights into the mechanisms that regulate or modulate heart rate in health and disease and provide a new perspective on the well-recognized association of heart rate with cardiovascular disease and mortality.

**URLs.** PANTHER, <http://www.pantherdb.org/>; Gene Ontology (GO), <http://www.geneontology.org/>; Molecular Signatures Database (MsigDB), <http://www.broad.mit.edu/gsea/msigdb/collections.jsp>; Mascot Search algorithm, <http://www.matrixscience.com>; Ensembl, <http://www.ensembl.org/index.html>; SNIPPER, <http://csg.sph.umich.edu/boehnke/snipper/>.

## METHODS

Methods and any associated references are available in the [online version of the paper](#).

*Note: Supplementary information is available in the online version of the paper.*

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#### COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the [online version of the paper](#).

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Müller-Nurasyid<sup>97,116,117</sup>, Pau Navarro<sup>32</sup>, Mari Nelis<sup>4,5,118</sup>, Jeffrey R O'Connell<sup>42</sup>, Christopher J O'Donnell<sup>110,119,120</sup>, Ken K Ong<sup>1,121</sup>, Anne B Newman<sup>122</sup>, Annette Peters<sup>98,123</sup>, Ozren Polasek<sup>124</sup>, Anneli Pouta<sup>88,125</sup>, Peter P Pramstaller<sup>90,126,127</sup>, Bruce M Psaty<sup>71,72,89,128,129</sup>, Dabeeru C Rao<sup>130</sup>, Susan M Ring<sup>113</sup>, Elizabeth J Rossin<sup>20,131–133</sup>, Diana Rudan<sup>134</sup>, Serena Sanna<sup>135</sup>, Robert A Scott<sup>1</sup>, Jaban S Sehmi<sup>102,136</sup>, Stephen Sharp<sup>1</sup>, Jordan T Shin<sup>8</sup>, Andrew B Singleton<sup>137</sup>, Albert V Smith<sup>85</sup>, Nicole Soranzo<sup>19,138</sup>, Tim D Spector<sup>138</sup>, Chip Stewart<sup>20,139</sup>, Heather M Stringham<sup>35,36</sup>, Kirill V Tarasov<sup>106</sup>, André G Uitterlinden<sup>3,91,140</sup>, Liesbeth Vandenput<sup>41</sup>, Shih-Jen Hwang<sup>110</sup>, John B Whitfield<sup>141</sup>, Cisca Wijmenga<sup>15</sup>, Sarah H Wild<sup>142</sup>, Goncke Willemsen<sup>52</sup>, James F Wilson<sup>142</sup>, Jacqueline C M Witteman<sup>3,91</sup>, Andrew Wong<sup>121</sup>, Quenna Wong<sup>54</sup>, Yalda Jamshidi<sup>138,143</sup>, Paavo Zitting<sup>144</sup>, Jolanda M A Boer<sup>145</sup>, Dorret I Boomsma<sup>52</sup>, Ingrid B Borecki<sup>53</sup>, Cornelia M van Duijn<sup>3,18</sup>, Ulf Ekelund<sup>1,146</sup>, Nita G Forouhi<sup>1</sup>, Philippe Froguel<sup>60,73,147</sup>, Aroon Hingorani<sup>104</sup>, Erik Ingelsson<sup>2,58,148</sup>, Mika Kivimaki<sup>104</sup>, Richard A Kronmal<sup>54</sup>, Diana Kuh<sup>121</sup>, Lars Lind<sup>149</sup>, Nicholas G Martin<sup>141</sup>, Ben A Oostra<sup>150</sup>, Nancy L Pedersen<sup>58</sup>, Thomas Quertermous<sup>101</sup>, Jerome I Rotter<sup>151</sup>, Yvonne T van der Schouw<sup>57</sup>, W M Monique Verschuren<sup>145</sup>, Mark Walker<sup>152</sup>, Demetrius Albanes<sup>75</sup>, David O Arnar<sup>153,154</sup>, Themistocles L Assimes<sup>101</sup>, Stefania Bandinelli<sup>155</sup>, Michael Boehnke<sup>35,36</sup>, Rudolf A de Boer<sup>31</sup>, Claude Bouchard<sup>47</sup>, W L Mark Caulfield<sup>16,17</sup>, John C Chambers<sup>44,102</sup>, Gary Curhan<sup>156,157</sup>, Daniele Cusi<sup>70</sup>, Johan Eriksson<sup>48,158–160</sup>, Luigi Ferrucci<sup>49</sup>, Wiek H van Gilst<sup>31</sup>, Nicola Glorioso<sup>161</sup>, Jacqueline de Graaf<sup>92</sup>, Leif Groop<sup>24</sup>, Ulf Gyllensten<sup>34</sup>, Wen-Chi Hsueh<sup>43</sup>, Frank B Hu<sup>28</sup>, Heikki V Huikuri<sup>96</sup>, David J Hunter<sup>162</sup>, Carlos Iribarren<sup>163</sup>, Bo Isomaa<sup>159,164</sup>, Marjo-Riitta Jarvelin<sup>44,45,125,165,166</sup>, Antti Jula<sup>167</sup>, Mika Kähönen<sup>168</sup>, Lambertus A Kiemeny<sup>169,170</sup>, Melanie M van der Klauw<sup>171</sup>, Jaspal S Kooner<sup>102,136</sup>, Peter Kraft<sup>162</sup>, Licia Iacoviello<sup>172</sup>, Terho Lehtimäki<sup>168</sup>, Marja-Liisa L Lokki<sup>173</sup>, Braxton D Mitchell<sup>42</sup>, Gerjan Navis<sup>174</sup>, Markku S Nieminen<sup>175</sup>, Claes Ohlsson<sup>41</sup>, Neil R Poulter<sup>176</sup>, Lu Qi<sup>28</sup>, Olli T Raitakari<sup>177,178</sup>, Eric B Rimm<sup>28</sup>, John D Rioux<sup>26,27</sup>, Federica Rizzi<sup>179</sup>, Igor Rudan<sup>142</sup>, Veikko Salomaa<sup>48</sup>, Peter S Sever<sup>176</sup>, Denis C Shields<sup>180–182</sup>, Alan R Shuldiner<sup>42,183</sup>, Juha Sinisalo<sup>175</sup>, Alice V Stanton<sup>184</sup>, Ronald P Stolk<sup>10</sup>, David P Strachan<sup>29</sup>, Jean-Claude Tardif<sup>26,27</sup>, Unnur Thorsteinsdottir<sup>13,154</sup>, Jaako Tuomilehto<sup>185–188</sup>, Dirk J van Veldhuisen<sup>31</sup>, Jarmo Virtamo<sup>48</sup>, Jorma Viikari<sup>189</sup>, Peter Vollenweider<sup>190</sup>, Gérard Waeber<sup>190</sup>, Elisabeth Widen<sup>191</sup>, Yoon Shin Cho<sup>192</sup>, Jesper V Olsen<sup>22</sup>, Peter M Visscher<sup>193</sup>, Cristen Willer<sup>194</sup>, Lude Franke<sup>15,195</sup>, Global BPgen Consortium<sup>196</sup>, CARDIOGRAM Consortium<sup>196</sup>, Jeanette Erdmann<sup>90,197,198</sup>, John R Thompson<sup>199</sup>, PR GWAS Consortium<sup>196</sup>, Arne Pfeufer<sup>200,201</sup>, QRS GWAS Consortium<sup>196</sup>, Nona Sotoodehnia<sup>71,202</sup>, QT-IGC Consortium<sup>196</sup>, Christopher Newton-Cheh<sup>12,110,203</sup>, CHARGE-AF Consortium<sup>196</sup>, Patrick T Ellinor<sup>62,204,205</sup>, Bruno H Ch Stricker<sup>3,91,103,140,206</sup>, Andres Metspalu<sup>4–6</sup>, Markus Perola<sup>5,39,48</sup>, Jacques S Beckmann<sup>37,207</sup>, George Davey Smith<sup>9</sup>, Kari Stefansson<sup>13,154</sup>, Nicholas J Wareham<sup>1</sup>, Patricia B Munroe<sup>16,17</sup>, Ody C M Sibon<sup>99</sup>, David J Milan<sup>8,208</sup>, Harold Snieder<sup>10</sup>, Nilesh J Samani<sup>209,210</sup> & Ruth J F Loos<sup>1,211–213</sup>

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## ONLINE METHODS

**Stage 1 genome-wide association meta-analysis.** The discovery sample encompassed 36 studies with data on heart rate in up to 85,787 individuals of European ancestry. Heart rate was derived from ECG in 12 studies (32% of the total sample) and peripheral pulse rate in 22 studies (49%) and was self-reported in 2 studies with data from health professionals (19%) (**Supplementary Table 1**). All studies included have been approved by local ethics committees, and all participants have provided their consent in writing.

Samples were genotyped using Affymetrix and Illumina genome-wide genotyping arrays (**Supplementary Tables 2 and 3**) and were imputed for polymorphic HapMap Phase 2 European CEU SNPs using MACH<sup>26</sup>, IMPUTE<sup>27</sup>, BAMBAM<sup>28</sup> or Beagle<sup>29</sup> (**Supplementary Table 2**).

Each study performed SNP association analyses with heart rate using an additive genetic model implemented in MACH2QTL<sup>30</sup>, Merlin<sup>31</sup>, SNPTEST<sup>27</sup>, ProbABEL<sup>32</sup>, GenABEL<sup>33</sup>, LME in R, MMAP, Matlab or PLINK<sup>34</sup> (**Supplementary Table 2**). Associations were adjusted for age, age<sup>2</sup>, body mass index (BMI) and study-specific covariates when appropriate (for example, principal components). Analyses were stratified by sex and case status for samples ascertained for diseases or conditions. To allow for relatedness in the deCODE, HAPI Heart, Heritage, Korcula, NBS, NSPHS and SPLIT studies, regression coefficients were estimated in the context of a variance component model that took into account relatedness in men and women, with sex as an additional covariate. Before meta-analysis of the results from the 36 GWAS, we excluded SNPs with poor imputation quality score ( $r^2$  hat  $\leq 0.3$  in MACH, proper\_info  $\leq 0.4$  in IMPUTE and BAMBAM, info  $\leq 0.8$  in Beagle), low minor allele count ( $n \times \text{MAF} \leq 3$ ) and/or extreme effect size ( $\beta > \pm 50$  bpm per effect allele, that is,  $\sim 5$  times the standard deviation of heart rate as typically observed in the contributing studies) in each sex- and case-specific stratum. Individual GWAS were corrected by genomic control before meta-analysis when appropriate ( $\lambda_{\text{GC}} > 1.000$ ) to adjust for population stratification.

We performed the stage 1 fixed-effects meta-analysis using the inverse variance method in METAL<sup>35</sup>. Before SNPs were selected for follow-up, a final genomic control correction of the meta-analysis results was performed ( $\lambda_{\text{GC}} = 1.106$ ), giving conservative association estimates.

Lead SNPs at 42 independent loci were selected for follow-up in stage 2 ( $P < 3 \times 10^{-5}$ ) (**Supplementary Table 5**). Loci were considered to be independent if pairwise  $r^2$  for LD was less than 0.2 and if they were separated by at least 1 Mb. We subsequently performed conditional analyses using summary statistics of stage 1 results<sup>14</sup> to examine whether any of the 42 loci contained secondary associations with heart rate that remained significant after adjusting for the association of the lead SNP ( $P < 5 \times 10^{-8}$ ). Before embarking on the follow-up analysis using all available data, we made sure that the inclusion of subgroups of the population did not affect the results (**Supplementary Table 4**).

**Stage 2 follow-up.** We tested for association of the 42 lead SNPs and 3 secondary associations in data from up to 88,823 individuals of European descent from 37 *in silico* replication studies with heart rate in stage 2. Heart rate was derived from ECG in 22 studies (57% of the total sample) and from peripheral pulse rate in 15 studies (43%) (**Supplementary Table 7**). GWAS data were available for up to 60,396 individuals of European descent from 2 sources: a previously reported meta-analysis of 15 GWAS for RR interval (RRgen Consortium)<sup>12</sup> and 12 GWAS that have not been described previously in this context (**Supplementary Tables 7–9**). Additional data were available for 24,334 individuals of European ancestry from 11 studies who were genotyped using the MetaboChip<sup>36</sup> and from 5,171 individuals of European descent from 1 study who were genotyped using the Cardiochip (**Supplementary Tables 8 and 10**).

Samples and SNPs that did not meet the quality control criteria described by each individual study and for stage 1 were excluded. Minimum genotyping quality control criteria were defined as Hardy-Weinberg equilibrium  $P > 1 \times 10^{-6}$  and call rate  $> 95\%$  in each of the follow-up studies.

We tested the association of the 42 lead SNPs and 3 secondary associations with heart rate in each stage 2 study separately as described for stage 1 studies. Missing SNPs in GWAS of stage 2 were replaced by one of up to three proxies selected a priori ( $r^2 > 0.8$ ) (if available). Prioritizing of proxies was based on (in order of importance) (i) availability on the MetaboChip and/or Cardiochip with  $r^2 > 0.8$ ; (ii)  $r^2$  for LD; and (iii) proximity to the lead SNP. This resulted

in the inclusion of three proxies in LifeLines2 and one proxy in ACTS, all of which had  $r^2 > 0.9$  with the lead SNP at the locus (**Supplementary Table 10**). None of the loci for which these proxies were included has association reaching  $P < 5 \times 10^{-8}$  after meta-analysis of stages 1 and 2 together.

We performed meta-analysis on summary statistics from the stage 1 meta-analysis and stage 2 studies using the weighted z-score method in data from up to 181,171 individuals (**Supplementary Table 5**). Genomic control-adjusted  $P$  values were used throughout stages 1 and 2 for GWAS. For studies with data from MetaboChip and Cardiochip, little evidence for population stratification was previously observed for associations with other cardiovascular and metabolic traits, and, hence, no correction of  $P$  values was applied.

For loci with secondary associations, the SNP with the lowest  $P$  value for association with heart rate after combined meta-analysis of stage 1 and 2 results was considered the most representative for the locus. For loci with confirmed associations after meta-analysis of stage 1 and 2 results, an estimate of the effect size was obtained by fixed-effects meta-analysis of summary statistics from the stage 1 meta-analysis and stage 2 studies with heart rate, using the inverse variance method.

**Additional analyses and functional follow-up experiments.** *Cumulative effects of confirmed loci and interindividual variation in heart rate.* To estimate the cumulative effect of the 21 heart rate-associated loci, we calculated the GPS in 5,053 adults from the LifeLines2 study and 4,000 12-year-old children from the ALSPAC study by summing the number of heart rate-increasing alleles carried by an individual at the lead SNP of each heart rate locus. The number of heart rate-increasing alleles ranged from 9 to 26 for the 19 available loci in LifeLines2 (data from rs6127471 and rs2340782 were not available) and from 10 to 29 for the 21 loci in ALSPAC.

We compared the explained variance ( $r^2$ ) between covariate-adjusted models with and without the GPS to assess the variance in heart rate that can be explained by these loci in the LifeLines2 and ALSPAC studies.

*Association analyses with related traits.* Associations between the 21 heart rate loci and related cardiovascular intermediates and endpoints were extracted from GWAS data of the CHARGE Consortium (PR duration)<sup>16</sup>, the QRS GWAS Consortium (QRS duration)<sup>17</sup> and the QT-IGC Consortium (QT duration; QT-IGC Consortium (C.N.-C.), personal communication) (**Table 2**), as well as from deCODE Genetics (prevalent advanced (second- or third-degree) atrioventricular block, SSS, pacemaker implantation and sudden cardiac death)<sup>13,18</sup> (**Table 2 and Supplementary Table 12**), deCODE Genetics<sup>13</sup> and the CHARGE-AF Consortium<sup>25</sup> (atrial fibrillation) (**Table 2**), the Global BPgen Consortium (systolic and diastolic blood pressure, as well as prevalent hypertension)<sup>21</sup> (**Supplementary Table 13**) and the CARDIoGRAM Consortium (prevalent CAD and myocardial infarction)<sup>22</sup> (**Supplementary Table 14**). All associations were adjusted for covariates as described previously<sup>13,16–18,21,22,25,37</sup>.

We calculated multi-SNP predisposition scores for each trait to examine the association of the 21 heart rate loci combined with each of the before-mentioned traits, on the basis of single-SNP summary statistics and weighting by effect sizes for association with heart rate after meta-analysis of stages 1 and 2 together.

Associations of the 21 heart rate loci, both individually and in combination in a multi-SNP predisposition score, with related traits were considered statistically significant at  $P < 0.002$ , that is,  $\alpha = 0.05$  with Bonferroni correction for 21 independent tests.

*Enrichment analysis of heart rate associations in biological pathways.* We used MAGENTA<sup>24</sup> to test whether predefined biological processes or molecular functions were enriched for multiple modest heart rate associations, aiming to discover new pathways associated with heart rate and to test whether the 21 heart rate loci cluster near genes that constitute specific biological connections (**Supplementary Tables 18 and 19**). First, we calculated a corrected gene association  $P$  value for each gene in the genome and grouped genes into pathways using annotations from the Kyoto Encyclopedia of Genes and Genomes (KEGG), BIOCARTEA, Protein Analysis THrough Evolutionary Relationships (PANTHER)<sup>38</sup>, Biological Processes (PANTHER, BP) and Molecular Functions (PANTHER, MF), REACTOME, Gene Ontology (GO)

and Ingenuity databases. Finally, for each pathway, we evaluated potential enrichment of highly ranked gene scores by comparing the fraction of genes within each gene set whose corrected *P* value was more significant than the 95th percentile of all gene *P* values to that of 10,000 randomly sampled gene sets of identical size from the genome<sup>24</sup>. In significantly enriched gene sets, in addition to genes in validated association regions, the top ranked genes above the enrichment cutoff may suggest new modest associations for follow-up (Supplementary Table 19).

**Proteomics experiments in mouse heart and genetic enrichment analysis.** We used results from proteomics experiments to identify genes located within the heart rate loci that are expressed at the protein level in mouse heart and that are phosphorylated upon stimulation of  $\beta$ 1AR (A.L., M.N. Andersen, A.B. Steffensen, H. Horn, C.D. Kelstrup *et al.*, unpublished data). Briefly, male C57BL/6 mice were either treated with  $\beta$ 1AR- and  $\beta$ 2AR-specific antagonists (control group, *n* = 3) or with a  $\beta$ 2AR-specific antagonist followed by  $\beta$ 1AR-specific agonist (test group, *n* = 3). Cardiac proteins were extracted and digested, enriched for phosphopeptides by TiO<sub>2</sub> chromatography and analyzed by nanoflow liquid chromatography tandem mass spectrometry, as described previously<sup>39</sup>.

A total of 8,518 phosphorylation sites that could be mapped to a specific residue were identified. Mice in the test and control groups were compared using a two-sided *t* test with permutation-based FDR < 0.01. The number of genes encoding proteins identified in the experiments was 4,096. Forty-one of these were located within 500 kb of confirmed heart rate associations, four of which were regulated by  $\beta$ 1AR stimulation (*MYH6*, *PLN*, *TTN* and *NCL*).

**eQTL analyses.** We examined associations between each of the heart rate loci and expression of genes in *cis* in 1,469 whole-blood samples (PAXgene), reflecting primary leukocyte gene expression<sup>40</sup> (Supplementary Table 21). Transcriptional components were applied to reduce a substantial proportion of interindividual non-genetic expression variation. An eQTL meta-analysis was subsequently performed on the residual expression variation. We used FDR < 0.05 to correct for multiple testing. We removed 50 principal components by linear regression to remove non-genetic variation in gene expression. In addition, we performed conditional analyses to examine to what extent each heart rate-associated SNP explains the association between the gene transcript and the SNP most significantly associated with the gene transcript.

Significant *cis* associations were observed between five heart rate-associated SNPs and the levels of nine nearby transcripts in blood (Table 1 and Supplementary Table 21). The heart rate-associated SNPs explained a substantial proportion of the association with the most significant SNP for the gene transcript in conditional analyses (adjusted *P* > 0.05) for *TRIP6*, *TMEM258* (*C11orf10*), *FADS1*, *BEST1-FTH1*, *FADS2*, *CEP85L* (*C6orf204*) and *NCL-SNORD20*.

**Potentially functional variants within the 21 loci.** To identify SNPs in the confirmed loci that may be causal for the association with heart rate, we explored whether the heart rate-associated SNPs were in strong LD ( $r^2 > 0.8$ ) with variants in transcription factor binding sites, nonsynonymous SNPs or copy number variants (deletion variants and mobile element insertion polymorphisms) identified in the 1000 Genomes Project CEU Pilot 1 or HapMap CEU reference panels<sup>41</sup>. For nonsynonymous SNPs, PANTHER<sup>38</sup> was used to assess whether the variant was likely to have a detrimental effect on protein function, based on alignment of evolutionarily related proteins (Supplementary Table 20).

One association tagged a variant in a transcription factor binding site near *CEP85L* (*C6orf204*; near *SLC35F1*). Nonsynonymous variants in strong LD with heart rate-associated SNPs were present in six genes (Supplementary Table 22), with the p.Arg1045Trp alteration encoded in *KIAA1755* likely having a deleterious effect on protein function<sup>38</sup>. Of interest, the rs180242 allele that was associated with lower heart rate tagged a common 723-bp deletion variant located 8 kb upstream of *GNG11* (ref. 42) (1000 Genomes Pilot ID P2\_M\_061510\_7\_474;  $r^2 = 0.96$ ) and was additionally associated with lower expression of *GNG11* in blood (Supplementary Table 21).

**Candidate genes based on the literature.** To identify additional candidate genes in the heart rate loci, we identified all genes within 500 kb of the 21 heart

rate-associated SNPs and performed an automated literature search using the search term 'heart' in the program SNIPPER (Supplementary Table 23). We identified many genes with established connections to embryonic cardiac development, cardiac conduction, cardiac contractile proteins, calcium regulation, angiogenesis and endothelial function (Supplementary Note). Many of the loci harbored genes in which mutations lead to dilated and hypertrophic cardiomyopathy (in *MYH6* and *MYH7*, *PLN* (near *SLC35F1*), *TTN* (near *CCDC141*), *MFN1* (near *GNB4*) and *CHRM2*).

**Experimental follow-up of positional candidate genes in *D. melanogaster* and *D. rerio*.** We used *D. melanogaster* and the zebrafish *D. rerio* as models to examine whether candidate genes within the heart rate loci were likely to underlie the associations identified by GWAS. Forty-nine positional candidate genes were identified on the basis of results from proteomics experiments and genetic enrichment analysis in mouse heart and eQTL analyses, as well as by the presence of functional variants and results from the automated literature search. In addition, we searched the genes located within 500 kb of associations for biological candidates (Supplementary Table 24).

**Experiments in *D. melanogaster*.** BLAST searches were performed to identify obvious *D. melanogaster* orthologs of positional candidate genes (Supplementary Table 25). We subsequently used RNAi<sup>43,44</sup> to downregulate orthologs of candidate genes (Vienna *Drosophila* RNAi Center). Expression of RNAi was induced by crossing with a *D. melanogaster* line expressing GAL4 driven by an actin promoter (stock 4414, Bloomington *Drosophila* Stock Center) (Supplementary Table 25).

*D. melanogaster* stocks were kept at 25 °C on standard medium. Pre-pupae were selected for tachypacing, an established *D. melanogaster* model for atrial fibrillation, as previously described<sup>45</sup>. We recorded videos through a microscope at 10 $\times$  magnification before and after tachypacing to visualize heart contractions in triplicate periods of 10 s. Heart rate was subsequently quantified using ImageJ software. An arrhythmia index was calculated as the ratio of arrhythmic periods and total measurement duration using the same software.

The number of positional candidate genes for which we analyzed results was reduced from 49 to 25 owing to the absence of orthologs (*n* = 13 genes), lack of RNAi lines (*n* = 3), non-viability of offspring (*n* = 1) and reduced viability, defined as the generation of fewer than 5 live offspring (*n* = 7) (Supplementary Fig. 6 and Supplementary Table 25). For the 25 remaining genes, represented by 23 orthologs, we compared heart rate and risk of arrhythmia in 11  $\pm$  5 (mean  $\pm$  s.d.) pupae with downregulated gene expression and 30 6000V controls. Ten additional pupae were available with downregulation of *stwl* (stonewall), a gene that is not anticipated to have a role in heart rate regulation and which can thus be interpreted as an extra control group. We targeted 13 orthologs using multiple independent RNAi lines.

We compared differences in heart rate between the offspring of RNAi-treated *D. melanogaster* and 6000V controls using a multilevel approach, adjusting for the dependence of repeated measures within pupae as a random effect. We compared RNAi lines with 6000V controls at baseline and after tachypacing. Differences in heart rate after tachypacing were examined with and without adjusting for average heart rate at baseline as a fixed effect. Results of analyses from multiple independent RNAi lines targeting the same ortholog were combined using the fixed-effects meta-analysis with inverse variance method (Supplementary Table 26).

We examined the risk of arrhythmia by comparing the number of arrhythmic cases and controls between RNAi-targeted orthologs and 6000V controls, both before and after tachypacing, using Fisher's exact test. Differences were considered statistically significant at *P* < 0.002, that is,  $\alpha = 0.05$  with Bonferroni correction for 23 independent tests.

**Experiments in *D. rerio*.** In the zebrafish *D. rerio*, we excluded genes in eight loci that mapped in or near positional candidate genes with extensive a priori evidence of a role in cardiovascular processes (Supplementary Table 27). A maximum of two positional candidate genes per locus were selected in the remaining loci, which together with a lack of zebrafish orthologs in 12 genes resulted in the selection of 12 positional candidate genes for follow-up in zebrafish experiments (Supplementary Fig. 6 and Supplementary Tables 25 and 27).

Wild-type *D. rerio* stocks from Ekwill Fish Farm were maintained using standard procedures. Morpholino oligonucleotides (GeneTools) were designed against orthologs of the 12 positional candidate gene primary transcripts targeting the first exon-intron boundaries, except for *tfpia* (the ortholog of *TFPI*), which was designed to target the intron 1–exon 2 boundary (Supplementary Table 27). Embryos were injected at the single-cell stage and were scored and analyzed 48 h later. Downregulation of candidate genes was confirmed using quantitative PCR (Supplementary Table 29).

Heart rate analysis was performed as previously described<sup>46</sup>. For each ortholog, we performed the procedure twice on different days, comparing heart rate in embryos from the same embryonic aliquot that were injected with either morpholino oligonucleotides or PBS. Heart rate was measured in  $26 \pm 4$  embryos injected with morpholino oligonucleotides and in  $27 \pm 5$  embryos injected with PBS.

Measures of heart rate alone do not provide information on cardiac contractility. We therefore additionally measured ventricular fractional shortening in a subsample of embryos ( $6 \pm 1$  embryos injected with morpholino oligonucleotides and  $6 \pm 2$  embryos injected with PBS) as previously described<sup>47</sup>.

Differences in heart rate and fractional shortening of the ventricular chamber were examined using linear regression and were adjusted for variation in the timing of the heart rate measurement.

For each group of embryos injected with morpholino oligonucleotides ( $42 \pm 18$  embryos), we assessed whether downregulation of positional candidate gene expression resulted in visible phenotypes that distinguished treated embryos from those injected with PBS.

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