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Genome-wide association study of PR interval

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The electrocardiographic PR interval (or PQ interval) reflects atrial and atrioventricular nodal conduction, disturbances of which increase risk of atrial fibrillation. We report a meta-analysis of genome-wide association studies for PR interval from seven population-based European studies in the CHARGE Consortium: AGES, ARIC, CHS, FHS, KORA, Rotterdam Study, and SardiNIA ($N = 28,517$). We identified nine loci associated with PR interval at $P < 5 \times 10^{-8}$. At the 3p22.2 locus, we observed two independent associations in voltage-gated sodium channel genes, *SCN10A* and *SCN5A*. Six of the loci were near cardiac developmental genes, including *CAV1-CAV2*, *NKX2-5* (*CSX1*), *SOX5*, *WNT11*, *MEIS1*, and *TBX5-TBX3*, providing pathophysiologically interesting candidate genes. Five of the loci, *SCN5A*, *SCN10A*, *NKX2-5*, *CAV1-CAV2*, and *SOX5*, were also associated with atrial fibrillation ($N = 5,741$ cases, $P < 0.0056$). This suggests a role for common variation in ion channel and developmental genes in atrial and atrioventricular conduction as well as in susceptibility to atrial fibrillation.

In myocardial excitation, the delay between the excitation of the atria and ventricles is determined by the sum of atrial and atrioventricular nodal conduction. This delay, measured in milliseconds, is reflected on the standard 12-lead electrocardiogram (ECG) by the PR interval or PQ interval. The PR interval has a substantial heritable component, with heritability estimates ranging between 30% and 50%[1–4].

Atrial fibrillation is the most common sustained arrhythmia and is independently associated with increased risk of stroke, heart failure, dementia and death[5]. Atrial fibrillation prevalence increases markedly with age, to nearly 9% in those 80–89 years of age, and is expected to triple by the year 2050 (ref. 6). Common genetic risk factors for atrial fibrillation[7] include variants on chromosome 4q25 near the *PITX2* gene[8], in 16q22.3 near the *ZFHX3* (*ATBF1*) gene[9], in 1q21 in the *KCNN3* gene (P.T.E., K.L.L., N.L. Glazer, A.P., A.A., M.K.C. *et al.*, unpublished results) and in 7q36.1 the K897T variant (rs1805123) of the *KCNH2* gene[10].

The PR interval is considered an intermediate phenotype for atrial fibrillation, as alterations in atrial action potential duration and in atrioventricular conduction influence both PR interval and atrial fibrillation risk[11]. Longitudinal data from the Framingham Heart Study (FHS) and the Atherosclerosis Risk in Communities Study (ARIC) demonstrate that PR interval prolongation is a predictor of increased atrial fibrillation risk[12,13]. In addition, PR interval prolongation has been shown in the FHS to be an independent predictor in a multifactorial risk score for atrial fibrillation predisposition[14].

We undertook a meta-analysis of genome-wide association studies (GWASs) to investigate the genetic determinants of the PR interval and their relationship to atrial fibrillation risk. Our goal was to identify genes that can provide insights into atrial disease and lead to new opportunities for atrial fibrillation prevention and therapy.

We studied individuals of European descent from seven community-based studies: the Age, Gene/Environment Susceptibility–Reykjavik Study (AGES)[15], ARIC[16], the Cardiovascular Health Study (CHS)[17], the FHS[18], the Kooperative Gesundheitsforschung in der Region Augsburg study (KORA)[19], the Rotterdam Study (RS)[20], and the SardiNIA study[3] (**Table 1** and Online Methods). Phenotypic data

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Table 1 Characteristics of participants in the seven community cohorts included in the meta-analysis of genome-wide association studies of PR

	AGES	ARIC	CHS	FHS	KORA F3	KORA S4	Rotterdam	SardiNIA
N, participants before exclusion	3,219	11,478	2,084	12,174[a]	1,644	1,100	5,271	4,305
N, participants after exclusion with genome-wide genotypes available[b]	2,471	6,486	1,769	7,636[a]	1,427	927	3,710	4,091
Sex, men, *N* (%)	922 (37.3)	2,977 (45.9)	773 (43.7)	3,511 (46.0)	728 (51.0)	447 (48.2)	1,541 (39.8)	1,782 (43.5)
Age, years, mean	76.1 ± 5.4	53.9 ± 5.7	72.9 ± 5.4	39.9 ± 10.3	61.6 ± 9.9	56.2 ± 7.1	67.8 ± 8.5	42.5 ± 17.1
PR interval, ms, mean	171.4 ± 27.7	162.7 ± 23.7	167.9 ± 28.5	152.1 ± 21.9	163.3 ± 23.3	164.6 ± 21.2	165.9 ± 24.1	154.2 ± 26.7
RR interval, ms, mean ± s.d.	925.9 ± 156.5	915.7 ± 132.4	949.1 ± 155.5	905.7 ± 174.8	963.5 ± 160.6	930.7 ± 139.2	861.9 ± 137.4	921.2 ± 152.2
BMI, kg/m^2, mean ± s.d.	27.1 ± 4.4	26.7 ± 4.7	26.5 ± 4.5	26.1 ± 4.9	28.0 ± 4.4	27.8 ± 4.5	26.1 ± 3.5	25.2 ± 4.6
Height, cm, mean ± s.d.	166.4 ± 9.1	168.8 ± 9.4	164.9 ± 9.6	169.1 ± 9.5	167.1 ± 9.1	167.4 ± 8.8	167.3 ± 9.3	160.1 ± 8.9
Systolic BP, mmHg, mean ± s.d.	143 ± 20	117.2 ± 16.3	137 ± 21	120 ± 15	134 ± 20	132 ± 19	139 ± 22	125 ± 18
Beta blockers (%)	780 (31.6)	Excluded	176 (10.0)	Excluded	283 (19.8)	99 (10.7)	Excluded	Excluded
Diuretics (%)	733 (29.7)	562 (8.7)	434 (24.5)	ND	273 (19.1)	76 (8.2)	292 (7.5)	45 (1.1)
Calcium antagonists[c] (%)	131 (5.3)	Excluded	78 (4.4)	Excluded	ND	ND	Excluded	Excluded
S.d. of PR residual after adjustment for covariates, ms	25.9	23.0	26.9	20.5	21.3	20.1	23.0	25.5

Exclusion criteria are given in **Supplementary Table 1**. BP, blood pressure; ND, not determined; *, non–dyhydropyridine calcium agonists.
[a]Samples are from the three generations of the Framingham Heart Study (G1 = 685, G2 = 3178, G3 = 3773). [b]The *N* used in the analysis. [c]Non-dihydropyridine calcium antagonists. S.d. of PR interval is given after adjustment for all covariateS.

including resting 12-lead electrocardiography, height, weight, systolic blood pressure and medication use were collected using standardized protocols in all studies. Exclusion criteria and covariates are described in **Supplementary Table 1.**

Study participants were genotyped using a variety of genome-wide SNP arrays. To facilitate comparison of results across studies, we imputed to the 2.5 million HapMap SNPs[21]. A recent review supports the validity of combining results across statistical and genotyping platforms[22]. Genotyping details, SNP quality-control filters and imputation methods for each study are summarized in **Supplementary Table 2**.

After exclusions, 28,517 individuals were available for study. The association of each SNP with the PR interval was adjusted for age, sex, RR interval, height, body mass index (BMI), systolic blood pressure and, in studies with multiple recruitment sites, study site. Studies adjusted for or excluded individuals using drugs known to alter the PR interval, including beta-blockers, diuretics and non–dihydropyridine calcium antagonists. Owing to restrictions imposed by institutional review boards at several of the study sites on the sharing of individual genetic data, it was not possible to perform analyses based on combined individual-level data. Therefore, we conducted inverse variance-weighted fixed-effects meta-analysis of the beta estimates from linear regression of PR interval. The coefficients, generated for each SNP, estimate the difference in PR interval per copy of the minor allele, adjusted for the covariates in the model. The genome-wide significance threshold was 5×10^{-8} (**Table 2**).

To determine whether there was an association between the PR-associated loci and atrial fibrillation risk, we conducted a meta-analysis of results from four studies of atrial fibrillation in subjects of European descent. The first was a study of 896 prevalent atrial fibrillation cases and 15,768 referents from the CHARGE cohorts[9]. The second was a study of 2,517 incident atrial fibrillation cases and 21,337 referents from the CHARGE cohorts. The third and fourth were independent case-control studies of prevalent atrial fibrillation: the German Competence Network on Atrial Fibrillation (AFNET; 2,145 cases and 4,073 controls)[10] and the Cleveland Clinic AF study (CCAF; 183 cases and 164 controls)[23] (**Table 3** and Online Methods). We performed an inverse-variance weighted meta-analysis of the logistic-regression results from the prevalent atrial fibrillation studies and the proportional hazards results from the incident atrial fibrillation study. The Bonferroni-adjusted significance threshold was $P = 0.05 / 9 = 0.0056$.

The study was performed in accordance with the Helsinki declarations and was approved by the local medical ethics and institutional review boards. All participants gave signed informed consent to use their DNA for genetic analyses.

The distribution of results from the meta-analysis of PR GWASs is summarized in **Figure 1**. In the quantile-quantile (Q-Q) plot there is a clear excess of extreme *P*-values (**Fig. 2**). Nine loci showed independent association signals with $P < 5 \times 10^{-8}$. We determined the genomic control factor (λ) for the linear regression analysis of PR interval to be 1.076 and report overall analysis results unadjusted for this λ value[24]. We did not observe evidence of heterogeneity in effect sizes for any of the nine loci (I^2 statistic, all $P > 0.05$; **Table 2**).

The strongest genome-wide association signal for PR interval was in chromosomal region 3p22.2. In this region we detected two association signals, one in *SCN10A* (rs6800541, $P = 2.1 \times 10^{-74}$) and the other in *SCN5A* (rs11708996, $P = 6.0 \times 10^{-26}$) (**Fig. 2** and **Table 2**).

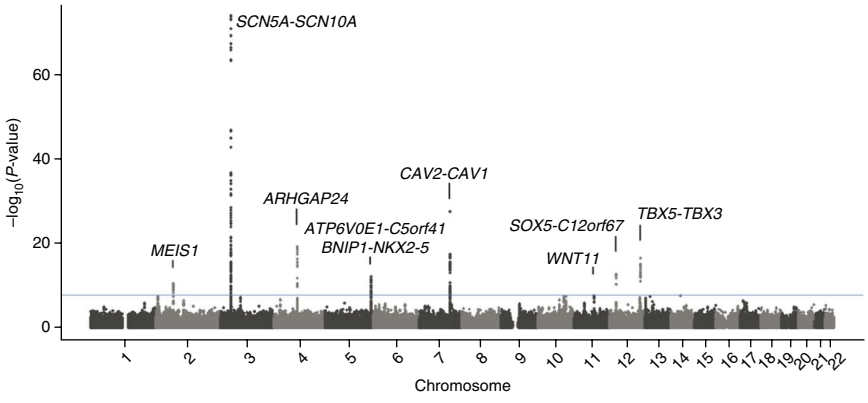

Figure 1 Manhattan plot of genome-wide association analyses. Genome-wide association results were combined across all studies by inverse variance weighting. The blue line marks the threshold for genome-wide significance ($P = 5 \times 10^{-8}$). Coordinates are given according to NCBI build 36.

These variants are in low linkage disequilibrium ($r^2 = 0.031$). In a meta-analysis of linear regression results from models including both SNPs, these SNPs remained independently associated with PR interval (rs6800541, $P = 9.7 \times 10^{-82}$; rs11708996, $P = 1.1 \times 10^{-33}$), suggesting they represent independent association signals.

SCN10A encodes the voltage-gated sodium channel Nav1.8, essential for cold perception in afferent nociceptive fibers of sensory dorsal root ganglia[25]. Nav1.8 is expressed in the peripheral sensory nervous system but has not been identified in the heart[26]. In *SCN10A*, two common nonsynonymous SNPs are in high to moderate linkage disequilibrium with the sentinel SNP: rs6795970 (V1073A, $r^2 = 0.933$) and rs12632942 (L1092P, $r^2 = 0.220$), making both SNPs good candidates to mechanistically explain this strongest PR-interval association identified in the human genome.

The neighboring *SCN5A* gene encodes Nav1.5, the main cardiac sodium channel, with mutations resulting in Brugada syndrome, long-QT syndrome, dilated cardiomyopathy, cardiac conduction disease, idiopathic ventricular fibrillation and atrial fibrillation[27].

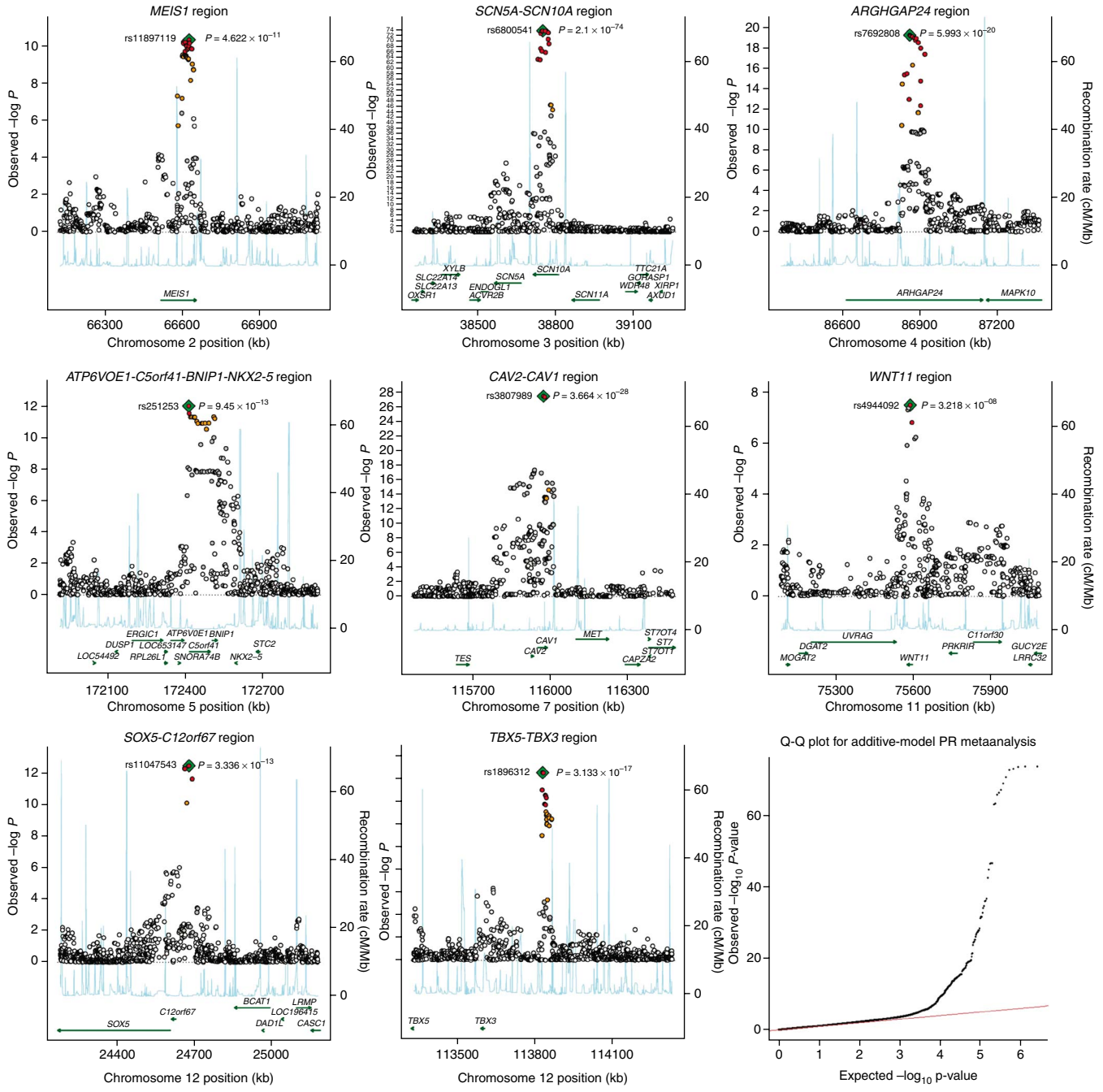

Figure 2 Association results at each significantly associated locus. Loci are displayed in genomic order from left to right: *MEIS1*, *SCN5A-SCN10A* region, *ARHGAP24*, *NKX2-5* region, *CAV1-CAV2* region, *WNT11*, *SOX5* region and *TBX5-TBX3* region. Each panel spans ± 500 kb around each SNP and has known gene transcripts annotated at the bottom. The SNPs are colored according to their degree of linkage disequilibrium (r^2), with the leading variant highlighted with a blue square and displayed by name and significance level achieved in the meta-analysis. Bottom right, Q-Q plot of the meta-analysis findings with a genomic control factor (λ) of 1.076.

Table 2 Genome-wide significant association findings for PR interval obtained at nine independent loci

Locus	SNP	Chr.	Position, build 36	Gene-related position	Minor/major allele	Method	RSQR (OEvar)	Freq. coded (minor) allele	Beta (ms)	S.e.m. (ms)	Association *P*-value	Heterogeneity: I^2 statistic	Heterogeneity: *P*-value
MEIS1	rs11897119	2	66625504	Intron 8	C/T	G	1.008	0.389	1.3624	0.207	4.62×10^{-11}	0	0.803
SCN5A	rs11708996	3	38608927	Intron 14	C/G	I	0.927	0.149	3.0403	0.2886	6.00×10^{-26}	0.045	0.396
SCN10A	rs6800541	3	38749836	Intron 14	C/T	G	0.995	0.404	3.7687	0.2065	2.10×10^{-74}	0.490	0.056
ARHGAP24	rs7692808	4	86860173	Intron 2	A/G	I	0.984	0.306	–2.0146	0.2203	5.99×10^{-20}	0	0.711
NKX2-5	rs251253	5	172412942	3 kb 5′ of *C5orf41*	C/T	G	0.971	0.399	–1.4924	0.2091	9.45×10^{-13}	0	0.926
CAV1-CAV2	rs3807989	7	115973477	Intron 2 of *CAV1*	A/G	G	0.970	0.395	2.2959	0.2086	3.66×10^{-28}	0	0.626
WNT11	rs4944092	11	75587267	Intron 1	G/A	G	1.006	0.321	–1.1916	0.2155	3.22×10^{-08}	0	0.549
SOX5	rs11047543	12	24679606	51 kb 5′ of *C12orf67*	A/G	I	0.983	0.147	–2.0907	0.2872	3.34×10^{-13}	0	0.912
TBX5-TBX3	rs1896312	12	113830807	226 kb 5′ of *TBX3*	C/T	I	0.936	0.279	1.9505	0.2311	3.13×10^{-17}	0	0.768

In each locus at least one marker exceeds the genome-wide significance threshold of $P < 5 \times 10^{-8}$. Beta values estimate the difference in PR interval per copy of the minor allele, adjusted for the covariates in the model. Chr., chromosome; freq., frequency. Column "Method" indicates whether a SNP was directly genotyped on one of the array platforms (G) or whether its genotype was imputed in all of the samples (I). RSQR (sometimes also termed OEvar) denotes the average of the observed by expected variance ratio of any SNP, which indicates deviation from Hardy-Weinberg equilibrium and quality of imputation. The RSQR and the minor allele frequency (MAF) are averages weighted by study size. All these SNPs met quality-control criteria in each study (**Supplementary Tables 2** and **3**). Effect size (beta) is reported in milliseconds (ms) per copy of the minor allele. We observed no heterogeneity in effect size estimates between studies and report the I^2 statistic results as the ratio of between-study to overall variance in beta values.

SCN5A sentinel SNP rs11708996 is in weak linkage disequilibrium with rs1805124 (H558R, $r^2 = 0.034$) as well as with two noncoding variants, rs12053903 ($r^2 = 0.030$) and rs11129795 ($r^2 = 0.058$), recently reported to be associated with QT interval[28,29]. This suggests that the PR-interval and QT-interval modifying effects are distinct.

Six PR-interval associations were identified in or near genes involved in human cardiac development (**Fig. 2** and **Table 2**). *NKX2-5* (rs251253, $P = 9.5 \times 10^{-13}$) is the homolog of the *Drosophila melanogaster tinman* gene and encodes the cardiac-specific homeobox transcription factor Nkx2.5 (Csx). Mutations can cause atrial septal defect with conduction defects (MIM108900), tetralogy of Fallot (MIM187500) and high-degree atrioventricular block[30]. In the *NKX2-5* gene region, the association extends over 200 kb and includes three other genes: *BNIP1*, *C5orf41* and *ATP6V0E1*. The signal at the *TBX5-TBX3* locus is 230 kb downstream of the paralogous *TBX5* and *TBX3* genes (rs1896312, $P = 3.1 \times 10^{-17}$). Both encode T-box-containing transcription factors important for cardiac conduction system formation in the developing heart[31]. *TBX5* is required for the patterning and maturation of the mouse atrioventricular and bundle branch conduction system[32]. Deletion of *TBX5* results in longer PR intervals in mice[33]. Mutations are seen in Holt-Oram syndrome (MIM142900), which includes atrial and ventricular septal defects, conduction disease, and occasionally atrial fibrillation[34]. *TBX3* controls formation of the sinus node and imposes pacemaker function on atrial cells[35]. Mutations cause ulnar-mammary syndrome (MIM181450), with limb, mammary, tooth, genital and cardiac abnormalities[36].

The *CAV1* and *CAV2* genes (rs3807989, $P = 3.7 \times 10^{-28}$) encode caveolins necessary for the development of caveolae involved in signal transduction[37]. *CAV1* is expressed in atrial myocytes. Mice deficient in Cav1 develop dilated cardiomyopathy and pulmonary hypertension[38]. *SOX5* (rs11047543, $P = 3.3 \times 10^{-13}$) and the nearby *C12orf67* encode transcription factors. *SOX5* knockout mice die with heart failure marked by hepatic congestion and peripheral edema[39]. *MEIS1* (rs11897119, $P = 4.6 \times 10^{-11}$) encodes a homeobox transcription factor implicated in cardiac, hematopoietic and neural development. *MEIS1*-deficient mice have malformed cardiac outflow tracts with overriding aorta and ventricular septal defect[40].

WNT11 (rs4944092, $P = 3.2 \times 10^{-8}$) encodes a signaling protein inducing cardiogenesis in *Xenopus laevis* and in mice by noncanonical Wnt signaling[41]. The nearest gene to a signal on chromosome 4 (rs7692808, $P = 6.0 \times 10^{-20}$) is *ARHGAP24*, which encodes a Rho-GTPase-activating protein and key angiogenic regulator that is involved in cell polarity, cell morphology and cytoskeletal organization[42] but without known relevance to the heart.

We tested for association of the nine identified PR loci with atrial fibrillation risk in 896 prevalent and 2,517 incident atrial fibrillation

Table 3 Characteristics of participants included in the meta-analysis of the nine PR loci and their association with atrial fibrillation

	CHARGE studies: prevalent AF analysis				CHARGE studies: incident AF analysis					Case control studies: prevalent AF analysis	
Baseline characteristics	AGES	CHS	FHS	RS	AGES	ARIC	CHS	FHS	RS	AFNET	CCAF
Participants											
N[a]	2,959	3,267	4,464	5,974	2,718	8,086	3,201	4,184	5,665	6,218	347
Sex, men, *N* (%)	1,154 (39.0)	1,278 (39.1)	2,004 (44.9)	2,427 (40.6)	1,011 (37.2)	3,814 (47.2)	1,241 (38.8)	1,830 (43.7)	2,282 (40.3)	3,569 (57.4)	202 (58.2)
Age[b], years, mean (s.d.)	76.5 (5.5)	72.3 (5.4)	65.5 (12.7)	69.4 (9.1)	76.3 (5.5)	57.0 (5.7)	72.2 (5.3)	64.7 (12.6)	69.1 (9.0)	52.7 (14.0)	56.4 (8.2)
Age[b], years, range	66–95	65–98	30–100	55–99	66–95	46–70	65–98	30–100	55–99	14–93	20–88
Hypertension, *N* (%)	2,260 (79.8)	1,711 (52.4)	2,263 (50.8)	1,997 (33.4)	2,145 (78.9)	2192 (27.1)	1,677 (52.4)	2,062 (49.4)	1,866 (32.9)	1,902 (30.6)	151 (43.5)
Cases											
AF cases	241	66	280	309	138	731	763	343	542	2,145	183
Age at AF onset, mean (s.d.)	76.9 (6.0)	NA	70.6 (10.6)	NA	80.6 (6.0)	67.0 (6.7)	81.2 (6.0)	77.4 (10.5)	77.7 (7.7)	59.7 (11.2)	46.0 (11.0)

AF, atrial fibrillation; NA, not available. Overall the CHARGE prevalent AF sample included 896 cases, the CHARGE incident AF sample included 2,517 cases and the case-control sample included 2,328 cases. [a]The *N* given is the number of participants in the CHARGE cohorts and the number of cases plus controls in case control studies. [b]Age was defined as age at DNA collection. Range gives the minimum and maximum ages.

Table 4 Meta-analytic association results for the nine PR loci significantly associated with atrial fibrillation

Nearest gene	SNP	Chr.	Position, build 36	PR prolonging allele	Frequency: PR prolonging allele	OR for AF: PR prolonging allele	OR for AF: 95% CI lower bound	OR for AF: 95% CI upper bound	*P* (unadjusted)	*P* (adjusted)	Effect of PR prolonging allele on AF risk	Effect of minor allele on AF risk
MEIS1	rs11897119	2	66625504	C	0.39	1.01	0.97	1.06	0.65	–	NS	NS
SCN5A	rs11708996	3	38608927	C	0.15	0.90	0.84	0.96	7.0×10^{-4}	6.30×10^{-3}	Decreased	Decreased
SCN10A	rs6800541	3	38749836	C	0.40	0.92	0.88	0.96	1.5×10^{-4}	1.35×10^{-3}	Decreased	Decreased
ARHGAP24	rs7692808	4	86860173	G	0.69	1.01	0.97	1.06	0.56	–	NS	NS
NKX2-5	rs251253	5	172412942	T	0.61	1.07	1.03	1.12	2.3×10^{-3}	2.07×10^{-2}	Increased	Decreased
CAV1/CAV2	rs3807989	7	115973477	A	0.40	0.91	0.87	0.95	2.2×10^{-5}	1.98×10^{-4}	Decreased	Decreased
WNT11	rs4944092	11	75587267	A	0.67	0.94	0.90	0.99	0.01	–	NS	NS
SOX5	rs11047543	12	24679606	G	0.85	1.13	1.06	1.20	2.1×10^{-4}	1.89×10^{-3}	Increased	Decreased
TBX5-TBX3	rs1896312	12	113830807	C	0.30	0.99	0.95	1.04	0.72	–	NS	NS

AF, atrial fibrillation; chr., chromosome; OR, odds ratio; CI, confidence interval; NS, not significant. Meta-analyzed were results from a meta-analysis of prevalent AF conducted in the CHARGE cohorts, results from a meta-analysis of incident AF conducted in the CHARGE cohorts, results from the AFNET case-control study and results from the CCAF case-control study. The Bonferroni-adjusted table-wide significance threshold is $P = 0.05 / 9 = 5.6 \times 10^{-3}$; the adjusted *P* column reports the significance thresholds after adjustment for nine tests. For all five SNPs we identified as associated with AF, the minor allele decreased the risk of AF irrespective of the direction of its effect on PR interval. Study-specific results are shown in **Supplementary Table 4**.

cases from the CHARGE Consortium plus 2,145 more cases from AFNET and 183 from CCAF (**Table 3**). Five of these loci showed association with atrial fibrillation risk at $P < 0.0056$. These were at *SCN10A* (rs6800541, $P = 1.5 \times 10^{-4}$) and *SCN5A* (rs11708996, $P = 7.0 \times 10^{-4}$), as well as three loci that include candidate genes with a known role in development—near the *NKX2-5* gene ($P = 2.3 \times 10^{-3}$), in the *CAV1-CAV2* genes ($P = 2.2 \times 10^{-5}$) and near the *SOX5* gene ($P = 2.1 \times 10^{-4}$). In all instances, the minor alleles were associated with a decrease in atrial fibrillation risk, irrespective of the direction of their association with PR interval (**Table 4**). Protective ratios against atrial fibrillation were between 0.93 and 0.88 for the minor alleles.

The observation that SNPs associated with both PR interval and atrial fibrillation risk did not show consistent directions of effect may initially seem counterintuitive. However, PR interval is an amalgamated measure of atrial and atrioventricular nodal conduction, which independently affect atrial fibrillation risk. PR intervals at both high and low extremes may be associated with an increase in atrial fibrillation risk. Indeed, existing data from humans and animal models suggest that the effects of genetic variants on atrial repolarization and action potential duration, and their relationships with atrial arrhythmias, are complex[11]. Both long and short QT intervals are associated with increased ventricular tachycardia risk. In analogy, assuming a linear association model for atrial fibrillation with PR interval and its underlying genetic variants may not capture the complexity of these relations.

Our study was subject to a number of potential limitations. False positive associations inherent in multiple testing are a limitation of any GWAS, so we used a well-accepted genome-wide association significance threshold equivalent to a Bonferroni correction for 1 million independent tests to reduce false positive findings[43]. Population stratification is also a concern, so we only included study subjects of European descent. The low genomic control inflation factor suggested there was no strong influence of population stratification on our results.

Our study also did not examine patterns of haplotype association. Thus, complex haplotype associations may not have been captured. However, genome-wide meta-analysis of haplotypes is not feasible at present, and, in common with other GWASs, our use of imputation to the HapMap leverages available linkage disequilibrium information.

The identification of *SCN10A* was unexpected, as Nav1.8 was previously not thought to have a role in cardiac electrophysiology. In addition, *SCN10A* was the only locus where two common nonsynonymous variants were in high linkage disequilibrium with a sentinel SNP and thus are likely causal candidates. The second key finding was that the majority of association signals we identified were in cardiac developmental genes, including two, *NKX2-5* and *TBX5*, in which mutations are known to cause well-defined cardiac malformations involving the atrial septum and the atrioventricular junction. The biological mechanisms by which the identified variants influence PR interval and atrial fibrillation remain speculative, and detailed functional investigation will be required to determine the potential contribution of each genomic region.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Participating cohort studies. *AGES.* The AGES study comprises a random sample of 30,795 men and women born between 1907 and 1935 living in Reykjavik in 1967 (ref. 15). For this study we investigated 3,219 participants who had been selected randomly and undergone genome-wide SNP genotyping. Phenotypic data were collected using standardized protocols; the clinic visit included a blood draw, blood pressure measurement, electrocardiography, anthropometry and measures of different domains of physical and cognitive function. White blood cells for DNA extraction were obtained, processed and stored. After the application of exclusion criteria, 2,471 probands were included in the GWAS analysis.

ARIC. The ARIC study includes 15,792 men and women from four communities in the United States (Jackson, Mississippi; Forsyth County, North Carolina; Washington County, Maryland; suburbs of Minneapolis, Minnesota) enrolled in 1987–1989 and prospectively followed[16]. ECGs were recorded using MAC PC ECG machines (Marquette Electronics) and processed initially by the Dalhousie ECG program in a central laboratory at the EPICORE Center (University of Alberta) but during later phases of the study using the GE Marquette 12-SL program (2001 version) at the EPICARE Center (Wake Forest University). PR interval was measured automatically, but in addition all ECGs were visually inspected for technical errors and inadequate quality. Of 11,478 participants aged 45–64 who described their race as white, 6,486 were part of the GWAS analysis after the application of exclusion criteria.

CHS. The CHS is a prospective, longitudinal cohort study of risk factors for cardiovascular disease in the elderly[17]. In 1989–1990 and 1992–1993, 5,888 participants 65 years of age or older were recruited from four field centers in the United States. Study electrocardiograms were recorded at baseline and processed as in the ARIC study in all clinical centers. There were $N = 2,084$ participants in the first two rounds of genome-wide genotyping who were without clinically recognized cardiovascular disease at baseline and who described their race as white. After the application of exclusion criteria, this analysis included $N = 1,769$ participants.

FHS. The FHS is a longitudinal observational, community-based cohort initiated in 1948 to prospectively investigate cardiovascular disease and its risk factors, as previously described[18]. For the present project, participants were eligible if they attended the eleventh examination of the original cohort and the first examination of the offspring and third-generation cohort. In FHS generations 1 and 2, digital caliper measurements were made of scanned paper ECGs recorded at 25 mm/s. PR interval duration was measured from the onset of the P wave to the onset of the QRS interval in two cycles of lead II by eResearch Technology. The coefficient of variation for the average of two measurements was 3.9%. In FHS generation 3, digital caliper measurements were made on scanned paper ECGs. PR interval duration was measured from the onset of the P wave to the onset of the QRS interval in two cycles of lead II, one cycle of lead V2 and one cycle of lead V5. For genome-wide association, PR and RR intervals were calculated as averages of two cycles in lead II. Within-person coefficient of variation was 4.1% for PR interval and 2.6% for RR interval.

KORA. KORA is a series of population-based epidemiological surveys of persons between 25 and 75 years old at the time of enrollment living in or near the city of Augsburg, Southern Germany[19]. KORA F3 was conducted between 2004 and 2005, and KORA S4 was conducted between 1999 and 2001. In 1.644 randomly selected individuals from F3 and 1027 from S4, genome-wide genotyping was performed. In KORA F3 and S4, 12-lead resting ECGs were recorded with digital recording systems (F3, Mortara Portrait; S4, Hörmann Medizinelektronik Bioset 9000). PR interval was measured automatically. In addition, all ECGs were visually inspected for technical errors and inadequate quality. The Mortara portrait determines PR intervals by a proprietary algorithm. PR intervals from Hörmann Bioset were determined using the Hannover ECG analysis software (HES version 3.22-12) by computerized analysis of an averaged cycle computed from all cycles of the 10-s recording after exclusion of ectopic beats. PR intervals determined by this algorithm represent the earliest beginning of atrial depolarization until the earliest deflection of ventricular depolarization between any two leads. In an international validation study, the HES software was among the best-performing digital ECG systems[44]. Reproducibility of HES measurements over short- and long-term time intervals has been investigated[45], and KORA data have been used in several GWASs of quantitative EKG traits[29,46].

RS. The Rotterdam Study (RS) is a community-based study founded in 1991 to examine the determinants of disease and health in older subjects with a focus on neurogeriatric, cardiovascular, bone and eye diseases[20]. Inhabitants of a suburb of Rotterdam, The Netherlands ($N = 10,275$) aged 55 years and older were invited, and 7,983 participants (78%) were examined. At baseline, 10-s, 12-lead ECGs were recorded at the research center with an ACTA Gnosis IV ECG recorder (EsaOte), stored digitally and analyzed with the Modular ECG Analysis System (MEANS). MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat. The PR interval is determined from the start of the P wave until the start of the QRS complex. The MEANS program has been evaluated and validated extensively[44,47]. MEANS is characterized by a high sensitivity (96.6%) and a high specificity (99.5%) in coding arrhythmias.

SardiNIA. The SardiNIA GWAS examined a total of 4,305 related individuals participating in a longitudinal study of aging-related quantitative traits in the Ogliastra province of the Sardinia region, Italy. The study has been described in detail previously[3]. Included individuals had four Sardinian grandparents and were selected without regard to their phenotypes. The ECG was recorded on paper (ECG machine Cardiette 600) with the participant at rest, and the PR interval was measured with ruler tracing in three consecutive beats from lead II.

Participating case-control studies. *AFNET.* AFNET is a German national registry of people with atrial fibrillation. In the context of the registry, DNA samples have been collected from individuals with atrial fibrillation onset before age 60 years at the Medical Department I of the University Hospital Munich, Campus Grosshadern of the Ludwig-Maximilians University Munich in collaboration with the Institute of Epidemiology at the Helmholtz Zentrum Munich. Cases were selected if the diagnosis of atrial fibrillation was made on an electrocardiogram analyzed by a trained physician. Subjects with signs of moderate to severe congestive heart failure or moderate to severe valve disease or with hyperthyroidism were excluded from the study. Referent subjects were drawn from the KORA S4 study, with ages ranging from 25 to 74 years, and had no history of atrial fibrillation, myocardial infarction, heart failure or valve disease and had documented sinus rhythm at the time of blood draw.

The KORA S4 study is a population-based epidemiological survey of persons living in or near the city of Augsburg, Southern Germany conducted between 1999 and 2001. The survey population consisted of German-nationality residents born between 1 July 1925 and 30 June 1975 identified through the registration office. A sample of 6,640 participants was drawn with ten strata of equal size according to sex and age, and 4,261 individuals (66.8%) agreed to participate.

CCAF. The Cleveland Clinic AF Study (CCAF) consists of subjects from the Cleveland Clinic Lone Atrial Fibrillation Gene Bank, which has enrolled patients with lone atrial fibrillation, defined as atrial fibrillation in the absence of clinically relevant structural heart disease. Subjects were at least 18 years of age and less than 66 years of age, with a history of recurring or persistent lone atrial fibrillation, ≤50% coronary artery stenosis in the coronary arteries (if cardiac catheterization done) or with normal stress test results (documentation of normal cardiac catheterization or stress test required if age ≥50 years), and had normal left ventricular ejection fraction ≥50%. Subjects were excluded if they had a heart failure, history of significant valvular disease (>2+ valvular regurgitation, any valvular stenosis), significant coronary artery disease (>50% coronary artery stenosis), previous myocardial infarction, previous percutaneous coronary intervention, coronary artery bypass graft, congenital heart disease, hypertrophic cardiomyopathy, aortic dissection or repair or latest left ventricular ejection fraction <50%. Referent subjects were drawn from the Gene Bank at the Cleveland Clinic Heart Center genotyped as part of an earlier case-control GWAS of myocardial infarction. Referent subjects were included if they had no history of atrial fibrillation at any age and otherwise met the inclusion criteria as described above for the early onset atrial fibrillation case cohort.

Genotyping. Genomic DNA was hybridized in accordance with the manufacturer's standard recommendations. Genotypes from Illumina arrays were obtained using the proprietary Bead Studio Software; genotypes from Affymetrix arrays were determined using the BRLMM[47] or Birdseed (see URLs) clustering

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algorithm. Each study performed filtering of both individuals and SNPs to ensure robustness for genetic analysis. SNP genotypes were assessed for quality, and SNPs failing quality control were removed before analysis according to specific criteria, as shown in **Supplementary Table 2**.

Genotype imputation. To facilitate the comparison of genotyping results across different platforms, genotypes from each study were imputed for the entire set of polymorphic HapMap SNPs (2.557 million). Imputation methods combined genotype data from each sample with the HapMap CEU samples[21] (July 2006, phased haplotypes, HapMap release 21) and then inferred unobserved genotypes probabilistically. The inference relies on the identification of shared haplotype stretches between study samples and HapMap CEU reference individuals. Most studies used a hidden Markov model as implemented in the MACH software (ref. 49; see URLs; for details, see **Supplementary Table 2**). In the MACH software, imputation results are summarized as an 'allele dosage' defined as the expected number of copies of one allele at that SNP (a fractional value between 0.0 and 2.0) for each genotype. In CHS, imputation was performed using BIMBAM[50] version 0.99 with reference to HapMap CEU using release 2, human genome build 36 and one round of imputations and the default expectation-maximization warm-ups and runs.

The measure RSQR (also termed OEvar) between each imputed genotype and the true underlying genotype was estimated by both MACH and BIMBAM as the average of the observed by the expected variance ratio of any SNP, which indicates deviation from Hardy-Weinberg equilibrium and quality of imputation and serves as a quality-control metric. We chose an estimated RSQR of ≤0.3 as a threshold to identify and discard low-quality imputations.

In SardiNIA, imputation was performed only in the 1,412 individuals genotyped with the Affymetrix Mapping 500K Array Set. For the remaining 2,893 individuals genotyped with the Affymetrix Mapping 10K Array, mostly offspring and siblings of the 1,412 individuals who were genotyped with the Affymetrix Mapping 500K Array Set, we took advantage of the relatedness among individuals to impute missing genotypes as described elsewhere[22,51]. Briefly, we identified large stretches of chromosomes shared within each family identical by descent and probabilistically filled in genotypes within each stretch whenever one or more of its carriers was genotyped with the 500K Array Set.

Exclusion criteria, adjustment of PR. Before genome-wide association analysis, PR interval was evaluated for significant nongenetic covariates across all studies to define a set of appropriate exclusion criteria and covariates and agree on a harmonized model of PR interval. If standard errors for these covariates were large compared to their effect size estimates ($P > 0.05$) in the majority of the studies, covariates were used as exclusion criteria. In all other cases, PR was adjusted for covariates. Exclusion criteria for all studies were atrial fibrillation, pacer or defibrillator implant, Wolf-Parkinson-White syndrome, third-degree atrioventricular block (complete heart block), history of heart failure, history of myocardial infarction, digitalis glycoside use and type 1 or type III antiarrhythmic drug use as well as extreme values of PR interval (**Supplementary Table 1**). All studies adjusted PR interval for sex, age, RR interval, systolic blood pressure, height and BMI (**Table 1**). Users of beta-blockers, diuretics or non–dihydropyridine calcium antagonists were excluded in some studies or adjusted for in other studies. Studies with more than one site also adjusted for site.

Association tests and meta-analyses. In each study, PR interval was linearly regressed on allele dosage (0–2, either measured directly or imputed), adjusting for covariates. The software used is described in **Supplementary Table 2**. In the FHS and SardiNIA studies, which included first- and second-degree relatives, regression analyses also allowed for relatedness. In the CHS, the regression was weighted to reflect case-cohort sampling probabilities. All studies have a genomic control parameter <1.11 (**Supplementary Table 2**). We accepted associations as genome-wide significant if they exceeded a significance level of $P < 5 \times 10^{-8}$, which corresponds to a Bonferroni-type multiple testing adjustment for 1 million independent tests, the estimate derived from the International HapMap project for the genome of Central Europeans (CEU)[11].

Meta-analysis combining the evidence from individual studies was performed using inverse variance weighting in METAL (see URLs). To avoid syntax errors, meta-analysis was performed twice, at different sites; identical results were obtained. We chose ± 500 kb around SNPs with identified association signals as a suitable interval to evaluate existing genes and association signals. This choice is supported by the genomic extension of the association signals which do not exceed ± 500 kb from the main SNP (as graphically displayed in **Fig. 2**).

URLs. Birdseed (part of Birdsuite), http://www.broad.mit.edu/mpg/birdsuite/; CHARGE Consortium information, http://depts.washington.edu/chargeco/; MACH Markov chain haplotyping package, http://www.sph.umich.edu/csg/abecasis/MaCH/; METAL software, http://www.sph.umich.edu/csg/abecasis/Metal/download/.

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