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Genetic Determinants of P Wave Duration and PR Segment

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Abstract

Background—The PR interval on the electrocardiogram reflects atrial depolarization and AV nodal delay which can be partially differentiated by P wave duration and PR segment, respectively. GWAS have identified a number of genetic loci for PR interval but it remains to be determined whether this is driven by P wave duration, PR segment or both.

Methods and Results—We replicated 7 of the 9 known PR interval loci in 16,468 individuals of European ancestry. Four loci were unambiguously associated with PR segment while the others were shared for P wave duration and PR segment. Next, we performed a genome-wide analysis on P wave duration and PR segment separately and identified five novel loci. SNPs in *KCND3* (P=8.3×10^{-11}) and *FADS2* (P=2.7×10^{-8}) were associated with P wave duration, whereas SNPs near *IL17D (*P=2.3×10^{-8}), in *EFHA1* (P=3.3×10^{-10}) and *LRCH1* (P=2.1×10^{-8}) were associated with PR segment. Analysis on DNA elements indicated that genome-wide significant SNPs were enriched at genomic regions suggesting active gene transcription in the human right atrium. Quantitative-PCR showed that genes were significantly higher expressed in the right atrium and AV-node compared to left ventricle (P=5.6×10^{-6}).

Conclusions—Genetic associations of PR interval appear to be mainly driven by genetic determinants of the PR segment. Some of the PR interval associations are strengthened by a directional consistent effect of genetic determinants of P wave duration. Through genome-wide association we also identified genetic variants specifically associated with P wave duration which might be relevant for cardiac biology.

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Keywords

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Introduction

Recent genome-wide association (GWA) studies on PR interval, derived from the surface electrocardiogram (ECG), have been instrumental for gaining novel insight into the biology of underlying conduction traits. These analyses have led to the discovery of 9 genetic loci in Europeans[1, 2], Asians[3] and African Americans[4, 5], some of which also confer an increased risk of atrial fibrillation. However, PR interval is the summation of P wave duration and PR segment, which are considered to reflect different aspects of the cardiac cycle. P wave duration reflects the electrical signals that propagate through the atria and can indicate atrial enlargement[6]. The PR segment corresponds to the period that electrical signals are delayed at the atrioventricular (AV) node, before it travels through the ventricular branches to induce cardiac depolarization and may be prolonged during AV nodal dysfunction. It remains to be determined whether the previously identified PR-interval variants are driven by P wave duration, PR segment, or by both. Understanding the origin of these genetic associations can further refine our understanding of atrial and AV-node function. The aims of current study are to (1.) determine the association of the genetic variants identified by previous PR interval studies with P wave duration and PR segment (2.) determine whether genome-wide analyses of the sub-traits (PR segment and P wave duration) can lead to the identification of novel SNP-associations.

Methods

Study samples

Participants of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study[7] and the Lifelines study cohort [8] were included. The primary objective of the PREVEND program was to prospectively investigate the natural course of increased levels of urinary albumin excretion (UAE) as an indicator of increased cardiovascular and renal risk in the general population.

LifeLines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 165,000 persons living in the North East region of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics. Both are community based cohort studies from the northern part of the Netherlands and have been approved by the review board of the University Medical Center Groningen. This study adheres to the principles expressed in the Declaration of Helsinki. All subjects provided written informed consent. Standard 12 lead ECGs in PREVEND and Lifelines were recorded using Cardio Perfect equipment (Welch Allyn Cardio Control, Delft, The Netherlands). Individuals were excluded from this analysis for the following reasons: atrial fibrillation, atrial flutter,

electronic pacemaker rhythm, complete AV-block, or Wolff-Parkinson-White syndrome. Also participants with extreme measurements (more than ±4SD from the mean) were excluded on a per phenotype basis. Myocardial infarction and medication were not, or minimally associated to durations of the P wave or PR segment in PREVEND, for this reason we did not adjust for these variables as previous analysis did[1]. After exclusions, there were 3,496 PREVEND and 12,972 Lifelines individuals with phenotype and genotype data contributing to the genotype-phenotype association analyses. Characteristics of participants are summarized in Table S1.

Genotyping

Genotyping for PREVEND and Lifelines was performed on the Illumina CytoSNP12 v2 chip. Samples were excluded based on call rates below 0.95, gender mismatch, duplicate discordance and genetic similarity (identity by state > 0.2). Population stratification was assessed by principal component analysis (PCA) over the sample correlation matrix, based on 16,842 independent (LD-pruned) SNPs. Samples were excluded when they diverged from the mean with at least 3 standard deviations (Z-score > 3) for the first 5 principal components. SNPs were excluded with a minor allele frequency of <0.01, call rate <0.95, or deviation from Hardy Weinberg equilibrium ($P < 1 \times 10^{-5}$). Genome-wide genotype imputation was performed using Beagle v. 3.1.0, using the NCBI build 36 of Phase II HapMap CEU data (release 22) as reference panel.

Statistical Analyses

Residuals of P wave duration and PR segment were calculated using general linear regression models to adjust for age, gender and body mass index (BMI). GWAS analyses of imputed SNPs were performed on the standardized residuals using an additive genetic model in PLINK (v.1.07). Test statistics from each cohort were then corrected for their respective genomic control inflation factor to adjust for residual population sub-structure. Meta-analysis was performed using the inverse-variance method. SNPs with MAF <1% (weighted average across cohorts) were removed.

The initial analysis considered variants to be independent if the pair-wise LD (r^2) was less than 0.1 and if they were separated by at least 1 MB; this was defined a ‘locus’. To test if there were multiple SNPs in the same loci independently associated with P wave duration and/or PR segment we repeated the primary association analysis for each trait whilst adjusting on the trait-specific genome-wide significant sentinel SNPs by adding the SNP dosages as covariates in the regression analyses. We then combined the association results again for each study by inverse variance weighting.

Similarly, to assess the association of selected SNPs (Genome-wide significant SNPs and literature SNPs) with PR interval, we performed association analyses in PREVEND and Lifelines and combined the results using the inverse-variance method.

Regulatory DNA

We used data available on DNAse I hypersensitivity sites (DHSs) from 349 human tissues and cell lines (GEO accession numbers GSE29692 and GSE18927) of the ENCODE

project[9] and Roadmap Epigenomics Program[10]. Hotspots were identified using the hotspot algorithm and peaks were called at 5% false discovery rate in a uniform manner, as previously described[11].

Aligned sequence reads (bed files) from 7 distinct histone modification assays in various human tissues were obtained from the Roadmap Epigenomics Project release 8. Only samples with matching input DNA samples were included. If replicate experiments were available we aggregated the sequence reads. MACS (v1.4) software was used to identify significant peaks (1×10^{-3}) using a fixed DNA fragment size of 146 [12]. Three samples could not be called with MACS due to inconsistencies in the original data. As a result, this data set included aligned sequence reads of 323 samples (Table S2).

For annotation of functional elements we included cardiac transcription factor data on Tbx3, Gata4 and Nkx2-5 from mouse heart[13]; p300 marks in human adult and fetal heart and RNAP2 from human fetal heart [14]; and Gata4, Mef2, Nkx2-5, Srf and Tbx5 from the atrial HL-1 cell line [15]. We used the called peak data from GEO (GSE35151, GSE32587 and GSE21529). Peaks from mice were lift-over to human using the UCSC Genome Browser liftOver tool with the options "-minMatch=0.1 -multiple" after extending the regions by 1kb [16]. Conversion of hg18 to hg19 was also performed using the UCSC LiftOver tool when appropriate.

Quantitative expression analysis of genes associated with P wave duration and/or PR segment

Wild-type FVB animals were sacrificed at the age of 6-8 weeks. Left ventricles, right atria and atrioventricular node / junction tissue were dissected in sterile PBS and pooled per tissue type in 3 groups of 7 samples. Total RNA isolation was performed using the MagNA Lyser instrument (product no. 03358976001; Roche Applied Science) with MagNA Lyser Green beads (product no. 03358941001, Roche Applied Science) and TRIzol Reagent (catalog no. 15596-026; Invitrogen) according to manufacturer's protocol. After quantification, 400ng of total RNA was treated with DNase I (18068-015, Invitrogen) to remove genomic DNA from the RNA sample. cDNA synthesis was performed using the SuperScript® II Reverse Transcriptase kit (catalog no. 18064-014, Invitrogen). Expression of different genes was assayed with quantitative real-time PCR using the Roche LightCycler 480 system. Primer sequences are listed in Table S3. Relative start concentration (N(0)) was calculated as previously described [17]. Values were normalized to Gapdh expression levels, differences in expression values were tested by 2-tailed t-tests. All animal work was approved by the Animal Experimental Committee of the Academic Medical Center, University of Amsterdam, and was carried out in compliance with Dutch government guidelines.

Results

We performed GWA studies with 2.3 Million imputed autosomal SNPs (HapMap 2 build 36 CEU panel) in the same individuals on P wave duration and PR segment. There was no evidence for inflation of test statistics at the final meta-analysis results, $\lambda_{\text{P-duration}} = 1.002$, $\lambda_{\text{PR-segment}} = 1.019$ (Figure S1). Although PR segment is a smaller interval (~44 ms) than P

duration (~113 ms) on the ECG, PR segment explains more of the PR interval (r2=0.66, P < 10^{-99}) than P wave duration (r2=0.30, P < 10^{-99}), due to greater variation in PR segment (Table S1).

First, we looked up the association of the known PR interval SNPs in the P wave duration and PR segment association results (Table 1, Table S4). All previously identified PR interval loci that were significant in this study also showed an association signal with PR segment (P<0.004). Loci containing genes *SCN5A/SCN10A, CAV1* and *SOX5* were also found to be associated with P wave duration; however none of the known PR interval loci were specific for P wave duration. The association of the *ARHGAP24* and *TBX3* SNPs were higher for PR segment (P=1.1×10^{-14}, P=1.2×10^{-9}, respectively) compared to the association with PR interval (P=9.9×10^{-12} and P=5.0×10^{-6}, respectively). All other PR interval associations decreased by separating PR interval into PR segment and P wave duration.

Secondly, the genome-wide meta-analysis identified a total of 93 SNPs in 3 loci to be significantly genome-wide associated (P<5.0×10^{-8}) with P wave duration and 184 SNPs in 7 loci to be genome-wide associated with PR segment (Table S5, Table S6). Summary statistics of all 2.3M SNPs are provided as supplemental material. We identified a secondary signal (rs2253017) on chromosome 13 to remain genome-wide associated with PR segment while adjusting for rs2798269 (Table 2, Figure 1 and Figure S2). One locus was shared among both phenotypes (the *SCN10A* locus). In total we identified 10 independent genetic variants to be genome-wide associated with P wave duration and/or PR segment. The variants explained on average 1.2% (P wave duration) and 3.1% (PR segment) of the phenotypic variation in Lifelines and PREVEND. All genome-wide top SNPs (sentinel SNPs) per independent locus were well imputed as shown in Table S4.

The Phenotype-Genotype Integrator at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/gap/phegeni) was queried for literature on the significant SNPs and SNPs in LD (r^2>0.6, Hapmap). The *FADS2* locus stands out, rs174577 (11q12), a SNP that has previously been associated with metabolic traits (Table S7).

SNPs in regulatory DNA

To provide insight into tissue specific regulatory DNA mechanisms influencing P wave duration and PR segment, we explored DNAse I hypersensitivity sites and histone marks [11]. We assigned the lowest P value of P wave duration and PR duration to each of the 2.3 M SNPs. We then compared the ratio of all SNPs with a P value below 5 × 10^{-8} in peaks of DNA elements to the ratio of all 2.3 M SNPs in DNA elements. We considered all available tissues/experiments. Enrichment was defined if tissues passed the threshold of Q3+1.5*IQR. (**Figure 2**). SNPs in all available human heart tissues of interest (right atrium left ventricle, fetal heart), atria, and a few other non-heart tissues were over-enriched for various DNA elements.

The genome-wide significant SNPs were annotated with various DNA elements that had been measured in the human or mouse heart. We found that all sentinel SNPs or SNPs in LD (r^2>0.8, 1000genomes) overlap with at least one DNase 1 hypersensitivity site of human

fetal heart. In Figure S3 we provide an overview of the sentinel SNPs in DNA elements to prioritize loci for experimental follow-up.

Candidate genes and gene expression

We prioritized candidate genes by searching for (1) protein coding gene nearest to the sentinel SNP, and any other protein coding gene within 10kb (11 genes); (2) we also considered genes containing a coding SNP in high LD (1000G EUR, r^2>0.8) with the sentinel SNP (1 gene, SCN10A). This analysis identified 11 candidate genes (Table 2) for the 10 independent, genome-wide significant, SNPs.

To test the hypothesis that we identified regions actively transcribed in the right atrium and AV-node, we performed qPCR's of the nearest gene to the sentinel SNP or any gene within 10kb. Using carefully dissected tissue samples from adult mouse hearts, we analyzed the expression of the candidate genes in atrial, ventricular and AV-nodal components. From this analysis it is first noteworthy that *Tbx3* expression was most highly expressed in the AV-node tissue samples, thus acting as a validation for the dissection procedure. Using qPCR we observed that 10 of the 11 candidate genes were expressed in left ventricle, right atrium and AV-node in mice. Notably, *SCN10A* transcripts were not reproducibly detectable in mice, also in line with a recent study of atrial gene expression using RNAseq [18]. All genes except *N6AMT2* (lower expression) and *ARHGAP24* showed significantly higher expression in atria or AV-node compared to the left ventricle (P<0.05, Figure 2). This number of genes (6) was also a significantly higher number than would be expected by chance ($P_{binomial}=5.56*10^{-6}$ at the 0.05 level of significance). *N6AMT2* is annotated to rs2253017 ($P=2.3\times10^{-8}$, PR segment), which is located between *IL17D* (−3.7kb) and *N6AMT2* (+2.2kb). In contrast to *N6AMT2*, *IL17D* is expressed higher in the AV-node and right atrium compared to the left ventricle, making it a more likely candidate gene.

Discussion

The P wave and PR interval on the ECG are important traits that have proven relationship with and predictive value for normal and abnormal heart rhythm, supraventricular arrhythmias, and conduction disturbances. We replicated 7 out of 9 loci that were previously associated with PR interval, and report that these are mainly driven by genetic variants affecting the PR segment, and to a lesser extent P wave duration. We also observed that PR segment explains more of PR interval than P wave duration, in support of a genetic investigation of P wave duration. By studying the specific sub-traits, we identified 5 novel independent genome-wide significant associations with PR segment or P wave duration. Variant near *IL17D/N6AMT2*, *EFHA1* and *LRCH1* were specifically associated with PR segment, whereas variants near *KCND3* and *FADS2* were specifically associated with PR duration.

We observed that genome-wide significant SNPs were specifically localized in chromatin marks and DNase I hypersensitive sites of the human fetal heart, compared to many other human tissues. This indicates that functionality of regulatory DNA elements may underlie some of the associations. The SNP in *SCN10A* (rs6801957) has already been studied and shown to affect a functional enhancer in the heart [14]. Genome-wide SNPs were also over-

enriched in cell type specific histone marks associated with active enhancers and promoters, H3K27ac and H3K4me1, of the human right atrium[19]. The repressive-state associated mark H3K9ac in the right atrium contained no genome-wide SNPs, and showed depletion compared to the other tissues. This finding suggests the regulatory mechanism to be mediated by gene activation and not repression.

Our expression analyses showed specific AV-nodal expression of Tbx3, which is in line with the association of rs10850409 near *TBX3* with PR-segment, reflecting the AV-node-dependent duration on the ECG. Tbx3 is a well-characterized transcription factor that is required for the development of many tissues. In the heart it allows the cells to acquire pacemaker properties and is required for the specification of the AV conduction system[20].

We identified novel associations with P wave duration and PR segment in the genomic regions 1p13.2, 11q12.2, 13q12.11 (containing 2 independent signals) and 13q14.13. In locus 13q12.11, *IL17D* was a strong candidate based on the expression analysis. To date, there has only been one study on *IL17D* which described the protein to be preferentially expressed in skeletal muscle, brain, adipose tissue, heart, lung, and pancreas, and to stimulate IL-6 and NF-κB dependent IL-8 production[21] relevant for heart function[22, 23]. Future studies should identify its potential role in the atria. In the same locus we identified another independent signal containing *EFHA1*, the exact function of the protein remains to be determined but a recent publication suggests a role in mitochondrial calcium handling [24]. In locus 11q12.2, *FADS2*, a member of the fatty acid desaturases has been previously associated with a multitude of lipids[25, 26], and a prominent role of lipid metabolism in the heart has been extensively studied [27]. In locus 1p13.2, *KCND3* encodes the Kv4.3 α-subunit that conducts the cardiac fast transient outward K+ current (Ito). This current is prominent in the repolarization phase of cardiac action potential. Several gain-of-function mutations have been recently been associated with Brugada syndrome and early-onset lone atrial fibrillation [28]. Finally, 13q14.13 contains *LRCH1;* no function has been assigned to the protein product of this gene.

Strengths and Limitations

This study is the first to investigate the differences in SNP associations between P wave duration and PR segment. The participants in this study are from the northern parts of the Netherlands and measurements have been performed in the same center, which ensures a homogenous population and reduces variation in assays. The novel genotype-phenotype associations were highly statistically significant but future efforts are required to understand the origin of these associations. Additionally, we note that the identified variants explained little of the phenotypic variation in the population, but anticipate that the distinct genotype-phenotype associations with PR-interval's sub-traits are more relevant to provide new biological insight of the heart's function [29]. In this study we were the first to combine as many as 672 experiments on histone modifications and DNase I hypersensitivity sites in human tissues for insight into the results of genome-wide analyses and thereby validating them. However, the analyses on DNA elements and gene expression in the right atrium might differ from the left atrial tissue which is thought to be the responsible tissue for AF vulnerability[18]. It is unknown how the identified polymorphisms or which polymorphisms in

LD have an effect on genes function causing the association. In-depth experimental studies are necessary to address the loci for their causal genes and the mechanisms on how genomic regions can affect the different aspects of P wave durations.

Conclusion

PR interval appears to be mainly genetically driven by PR segment but some of the associations are further strengthened by a directional consistent effect of genetic determinants on P wave duration. By GWA of the sub-phenotypes, we also identified two novel SNPs specifically associated with P wave duration which may be relevant for atrial biology and three novel SNP associations for PR segment. Analysis on DNA elements indicated that we identified regions actively transcribed in the right atrium, which was further validated by expression profiling of the candidate genes. In conclusion, analyzing sub-phenotypes of the electrocardiogram can lead to the identification of novel loci and genes and therefore might provide new insights into cardiac biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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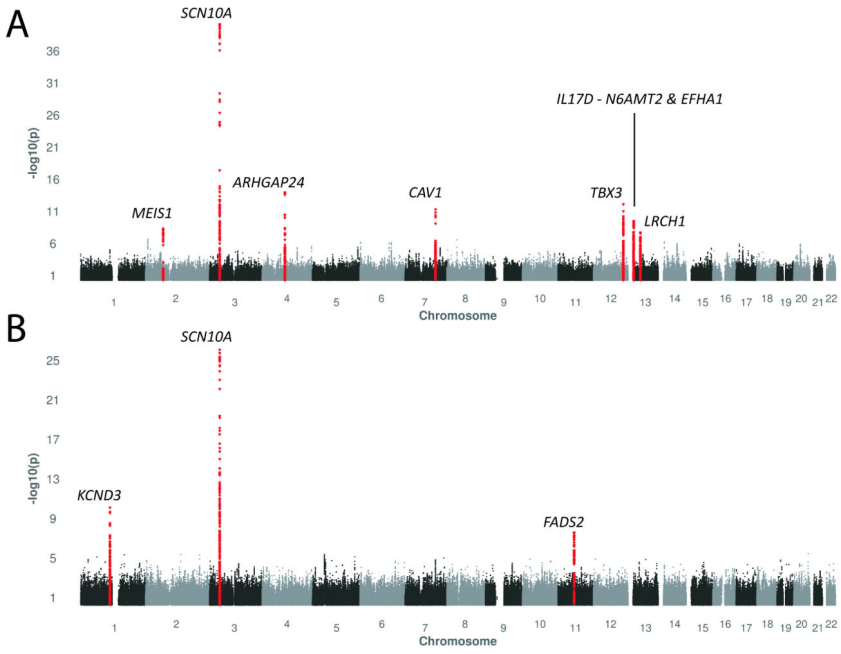

Figure 1.

Manhattan plots of the association of SNPs with a) PR segment and b) P wave duration. The *x*-axis represents the chromosomal position for each SNP; the y-axis represents the −log10 of the P-value for association. Three loci were significant for P wave duration and 7 loci were significant for PR segment.

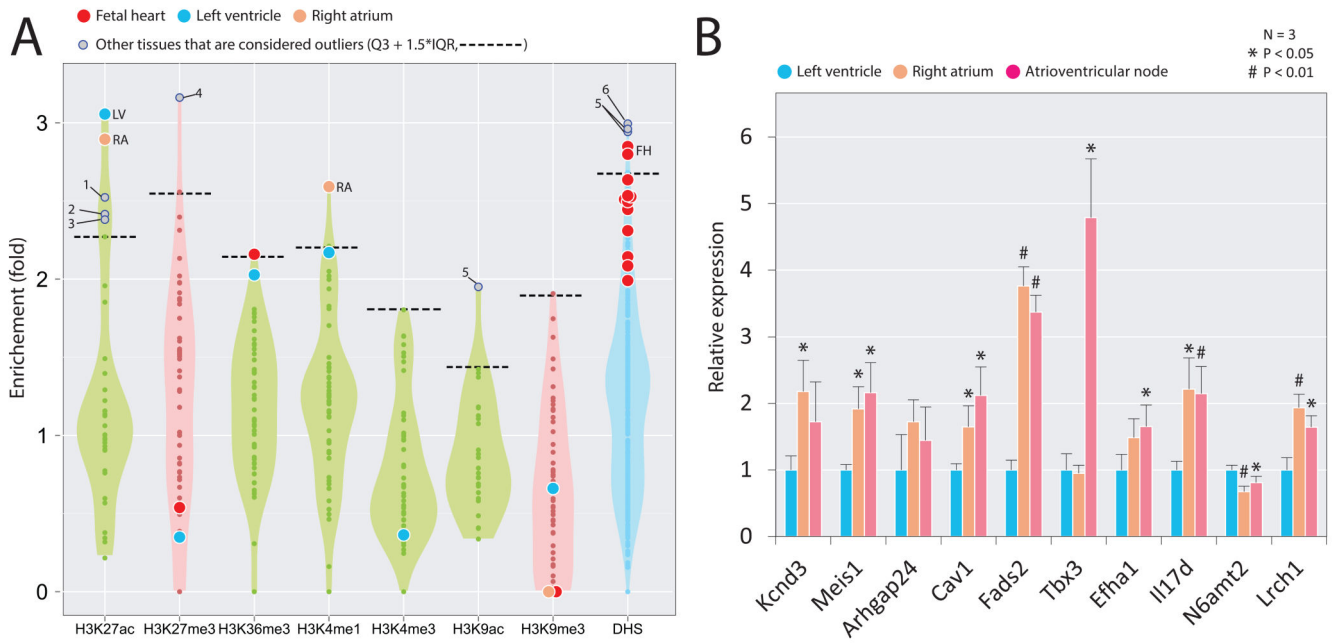

Figure 2 A.

Enrichment of SNPs in human tissues was tested in marks of H3K27ac (n=34), H3K27me3 (n=55), H3K36me3 (n=55), H3K4me1 (n=50), H3K4me3 (n=50, H3K9ac (n=26), H3K9me3(n=53) and DHS (n=349). Genome-wide significant SNPs were over-enriched in the active histone marks H3K27ac and H3K4me1 of the right atrium. In contrast, genome-wide significant loci were under-enriched in H3k9me3 of the right atrium, a mark that is associated with low levels of gene transcription. These results indicate that genome-wide significant loci are more active in the right atrium compared to other tissues, as expected. Furthermore, all available heart tissues were at least ≥1 time considered to be over-enriched, as well as a few other tissues such as aorta, skeletal muscle and fibroblasts. **B)** To test the hypothesis that we identified regions actively transcribed in the right atrium and AV-node, we performed qPCR's of the nearest genes identified in the genome-wide analysis of this study in the right atrium, AV-node and left ventricle. Three groups of 7 pooled tissue samples were measured for the expression of each gene and were normalized to the expression of Gapdh. Error bars represent the standard deviation. Significance of difference (* and #) between the left ventricle and the right atria or AV-node was tested using a t-test. Six of the 11 tested candidate genes were significantly higher expressed in both the node and right atrium when compared to the left ventricle. This is a significantly higher number than would be expected by chance ($P_{binomial}$ =5.56*10^{-6} at the 0.05 level of significance). LV=Left Ventricle, RA=Right atrium, FH=Fetal Heart, AVN=atrio-ventricular-node 1) Penis foreskin fibroblast primary cells, 2) Stomach, smooth muscle, 3) Aorta, 4) Brain anterior caudate, 5) Human skeletal muscle myoblasts, 6) Human mammary fibroblasts

Table 1

Seven of the nine known genome-wide PR interval loci [1-5] reached the threshold of significance for replication ($P < 0.05/9$) and were directionally concordant with previous reports, as indicated by bold P values and effect estimates. All loci that were significant for PR interval in this analysis were significantly associated with PR segment. However, SCN5A/SCN10A, CAV1 and SOX5 also showed an association with P wave duration. PR interval statistics were obtained in PREVEND and Lifelines and combined using the inverse-variance method. Frequencies were calculated from the individuals of Lifelines and PREVEND that were included in the meta-analyses. SNPs were pruned on LD ($r^2>0.6$, HapMap). Beta values estimate the difference in duration in millisecond per copy of the coding allele, adjusted for the covariates in the model.

			P wave duration GWAS		PR segment GWAS		PR interval			
Region	**SNP**	**Coding(FRQ) / non-coding allele**	**P-value**	**Beta(s.e.)**	**P-value**	**Beta(s.e.)**	**P-value**	**Beta(s.e.)**	**Ref**	**Gene**
2p14	rs3891585	A(0.41)/G	0.024	0.29(0.13)	2.30E-08	1.16(0.21)	**1.75E-09**	**1.44(0.24)**	1, 4, 5	MEIS1
3p22.2	rs267567	A(0.47)/G	0.781	−0.04(0.14)	0.320	0.22(0.23)	0.836	0.05(0.26)	5	ITGA9
3p22.2	rs3922844	T(0.33)/C	1.78E-08	−1.41(0.25)	3.79E-04	−1.46(0.41)	**4.12E-10**	**−2.96(0.47)**	4, 5	SCN5A
3p22.2	rs6599222	T(0.84)/C	6.80E-08	−1.98(0.37)	0.008	−1.61(0.60)	**6.28E-08**	**−3.74(0.69)**	4	SCN5A
3p22.2	rs6763048	NA	NA	NA	NA	NA	NA	NA	5	SCN5A
3p22.2	rs6801957	T(0.43)/C	8.44E-27	1.44(0.13)	6.94E-41	2.93(0.22)	**9.99E-69**	**4.41(0.25)**	1-5	SCN10A
4q21.23	rs7660702	T(0.70)/C	0.761	0.04(0.14)	1.14E-14	1.79(0.23)	**9.87E-12**	**1.82(0.27)**	1, 2, 4, 5	ARHGAP24
5q35.2	rs251253	T(0.59)/C	0.078	0.22(0.12)	3.56E-05	0.84(0.20)	**1.06E-05**	**1.04(0.24)**	1	ATP6V0E1, C5orf41
7q31.2	rs3807989	A(0.43)/G	3.50E-05	0.51(0.12)	4.99E-12	1.40(0.20)	**5.78E-16**	**1.89(0.23)**	2, 5	CAV1
11q13.5	rs4944092	A(0.76)/G	0.927	0.03(0.33)	0.956	−0.03(0.53)	0.796	0.16(0.61)	1	WNT11
12p12.1	rs11047543	A(0.14)/G	4.93E-04	−0.65(0.19)	1.57E-05	−1.32(0.31)	**3.44E-08**	**−1.94(0.35)**	1	SOX5
12q24.21	rs3825214	A(0.78)/G	0.031	−0.32(0.15)	0.287	−0.26(0.25)	0.029	−0.62(0.28)	2, 4, 5	TBX5
12q24.21	rs1896312	T(0.65)/C	0.231	0.19(0.16)	1.17E-09	−1.60(0.26)	**5.01E-06**	**−1.39(0.30)**	1	TBX3

Table 2

Summary statistics for the sentinel SNPs that were identified in the genome-wide association study on P wave duration and/or PR segment. P-values in bold indicate genome-wide associations. Beta values estimate the difference in duration in millisecond per copy of the coding allele, adjusted for the covariates in the model.

			P wave duration		PR segment			
Region	**SNP**	**Coding(FRQ)/ non-coding**	**P-value**	**Beta(s.e.)**	**P value**	**Beta(s.e.)**	**Ref**	**Gene**
1p13.2	rs2798334	T(0.29)/C	**7.92E-11**	−0.92(0.14)	0.002	−0.72(0.23)		KCND3
2p14	rs11678354	A(0.36)/T	0.010	0.34(0.13)	**4.71E-09**	1.26(0.22)	1, 4, 5	MEIS1
3p22.2	rs6801957	T(0.43)/C	**8.44E-27**	1.44(0.13)	**6.94E-41**	2.93(0.22)	1-5	SCN10A
4q21.23	rs13137008	T(0.70)/G	0.638	0.07(0.14)	**1.12E-14**	1.76(0.23)	1, 2, 4, 5	ARHGAP24
7q31.2	rs3807989	A(0.43)/G	3.50E-05	0.51(0.12)	**4.99E-12**	1.40(0.20)	2, 5	CAV1
11q12.2	rs174577	A(0.33)/C	**2.59E-08**	−0.73(0.13)	0.203	−0.28(0.22)		FADS2
12q24.21	rs10850409	A(0.27)/G	0.097	−0.23(0.14)	**7.62E-13**	1.65(0.23)	1	TBX3
13q12.11	rs2253017	T(0.15)/C	0.963	−0.01(0.17)	**2.20E-08**	1.58(0.28)		IL17D, N6AMT2
rs2253017 adjusted for rs2798269			-	-	**1.27E-08**	1.59(0.28)		
13q12.11	rs2798269	T(0.40)/C	0.923	−0.01(0.13)	**3.22E-10**	−1.38(0.22)		EFHA1
13q14.13	rs9590974	A(0.65)/C	0.243	−0.15(0.13)	**2.00E-08**	−1.19(0.21)		LRCH1