Genetic Determinants of Electrocardiographic P-wave Duration and Relation to Atrial Fibrillation

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Abstract

Background: The P-wave duration (PWD) is an electrocardiographic (ECG) measurement that represents cardiac conduction in the atria. Shortened or prolonged PWD is associated with atrial fibrillation (AF). We used exome chip data to examine the associations between common and rare variants with PWD.

Methods: Fifteen studies comprising 64,440 individuals (56,943 European, 5,681 African, 1,186 Hispanic, 630 Asian), and ~230,000 variants were used to examine associations with maximum PWD across the 12-lead ECG. Meta-analyses summarized association results for common variants; gene-based burden and SKAT tests examined low-frequency variant-PWD associations. Additionally, we examined the associations between PWD loci and AF using previous AF GWAS.

Results: We identified 21 common and low-frequency genetic loci (14 novel) associated with maximum PWD, including several AF loci (*TTN*, *CAND2*, *SCN10A*, *PITX2*, *CAV1*, *SYNPO2L*, *SOX5*, *TBX5, MYH6, RPL3L*). The top variants at known sarcomere genes (*TTN, MYH6*) were associated with longer PWD and increased AF risk. However, top variants at other loci (e.g., *PITX2* and *SCN10A*) were associated with longer PWD but lower AF risk.

Conclusion: Our results highlight multiple novel genetic loci associated with PWD, and underscore the shared mechanisms of atrial conduction and AF. Prolonged PWD may be an endophenotype for several different genetic mechanisms of AF.

Keywords: Exome-chip analysis, P-wave duration, atrial fibrillation, cardiac conduction

Non-standard Abbreviations and Acronyms

AF: atrial fibrillation

cMAC: cumulative minor allele count

GWAS: genome-wide association studies

LV: left ventricle

MAF minor allele frequency

PWD: P-wave duration

RAA: right atrial appendage

SKAT: sequence kernel association test

P-wave duration (PWD) is an electrocardiographic measurement that reflects cardiac conduction through the atria. PWD variability may implicate intrinsic or acquired properties in the function and structure of atrial conductivity.[1] Shortened and prolonged PWD have been repeatedly associated with atrial fibrillation (AF),[2, 3] a common and heritable[4] arrhythmia that predisposes to stroke, heart failure, and increased mortality.[5-7]

Although PWD is heritable[8, 9] only two genome-wide association studies (GWAS) have been conducted.[10, 11] Given the relationship between PWD and AF, examining the genetic determinants of PWD may provide insights into the pathophysiology of AF. Moreover, assessment of coding variation may facilitate identification of AF-specific genes. Therefore, we conducted an exome-chip based analysis focused on rare and common genetic determinants of PWD.

Methods

Each study was reviewed and approved by the local or institutional IRB, and each participant provided consent. Study-specific details are provided in **Supplemental Material**, under "Description of participating studies" and in **Supplemental Table 1**. In our primary analysis, we considered loci/genes significantly associated with PWD if a common variant (minor allele frequency [MAF] ≥ 5%) or a gene-based test, including burden or sequence kernel association test [SKAT][12] comprising low-frequency variants [MAF < 5% or MAF <1%]) exceeded exome-wide significance in meta-analyses, after Bonferroni correction. We reported low-frequency variants that exceeded exome-wide significance at significant loci identified in gene-based analyses. The full Methods section is available in the **Supplemental Material** (under

54 "Methods"). Data supporting the findings of this study can be made available, following
55 reasonable request to the corresponding author.
56
57 **Results**
58 A total of 64,440 individuals from 4 ethnic groups (56,943 European, 5,681 African, 630 Asian,
59 1,186 Hispanic) and 15 studies were included in our meta-analysis. The per-study mean age
60 ranged from 46.2-72.6 years; roughly 60% of participants were women (**Table 1**). For the multi-
61 ethnic single variant analyses, we tested ~26,000 common variants (see **Supplemental Table 3**
62 for the exact number of variants included in each analysis). The Quantile-Quantile plots show a
63 small degree of inflation for both PWD residuals (λ=1.10) and inverse normal transformed PWD
64 residuals (λ=1.13; **Supplemental Figures 1a-1b**). We performed meta-analyses in ethnicity-
65 specific groups (European: λ=1.10-1.13; African: λ=1.03; **Supplemental Figures 1c-1f**). LD score
66 regression intercepts were 1 (multi-ethnic analyses) and 0.95 (European-specific analyses),
67 suggesting the inflation was mainly due to polygenicity. Meta-analysis results from PWD
68 residuals, and inverse normal transformed PWD residuals were highly correlated across
69 analyses (Pearson's rho≥0.99, $P<2.2\times10^{-16}$; **Supplemental Figure 2).**
70
71 *Common variant analyses*
72 We identified 41 exome-wide significant variants at 18 loci (P-value $<1.9\times10^{-6}$; **Supplemental**
73 **Figure 3**) in our multi-ethnic meta-analysis of PWD residuals (**Table 2**). Eleven of the 18 PWD
74 loci are novel, representing the following nearest genes: *PKP1* (rs1626370, $P=2\times10^{-6}$), *TTN*
75 (rs2042995, $P=4\times10^{-7}$), *PITX2* (rs17042171, $P=8\times10^{-11}$), *ARHGAP10* (rs6845865, $P=2\times10^{-10}$),

TCF21 (rs2327429, $P=2\times10^{-7}$), *CDK6* (rs2282978, $P=2\times10^{-8}$), *SYNPO2L* (rs3812629, $P=4\times10^{-7}$), *SOX5* (rs17287293, $P=3\times10^{-7}$), *HMGA2* (rs8756, $P=7\times10^{-7}$), *GORS4* (rs17608766, $P=9\times10^{-15}$), and *MC4R* (rs12970134, $P=1\times10^{-6}$). Another novel locus was associated only with the inverse normal transformed PWD (*JAZF1*, $P=1\times10^{-6}$; **Table 2**; **Supplemental Table 4)**. The PWD variance explained by each of the top variants ranged from 0.04% to 0.44%; the top variants in aggregate explained ~1.6% of the phenotypic variance. Associations for *SCN10A* and *PITX2* regions were moderately heterogeneous across individual studies ($I^2 \geq 45\%$; **Table 2**). Of these 19 multi-ethnic significantly associated loci, 13 were significantly associated with PWD residuals in the European ancestry subset, and one (*SCN10A*) was observed in individuals of African ancestry (**Supplemental Table 4**). No additional loci were observed in analyses restricted to either European or African ancestry (**Supplemental Figure 4** for Manhattan plots).

In conditional analyses, we identified additional signals from *SCN5A* and *SCN10A* (**Supplemental Table 5)**. For inverse normal transformed PWD residuals, an additional signal (rs10033464, P-value=2×10^{-7}) was observed in the *PITX2* region. In addition to the 7 previously known loci that exceeded exome-wide significance, we observed 2 nominally significant associations with PWD at *SSBP3* and *EPAS1* (P <0.001; **Supplemental Table 6**).[10]

Gene-based analyses

We performed burden and SKAT tests for associations with PWD for 16,949 genes with a cumulative minor allele count (cMAC) ≥10, including 192,455 low-frequency and rare variants, in the multi-ethnic sample. We identified 4 genes associated with PWD using SKAT tests aggregating functional variants with MAF <5% (*TTN*, $P=6\times10^{-27}$; *DLEC1*, $P=2\times10^{-13}$; *SCN10A*,

98 P=7×10^{-8}; and *RPL3L*, P=9×10^{-7}; **Table 3**). We identified an additional association (*TTC21A,*
99 P=1×10^{-6}) using inverse normal transformed PWD residuals in the European-specific analysis.
100 Using burden tests, we identified *TTN* and *MUC5B* as PWD-associated genes in the multi-ethnic
101 and European-specific analyses. We did not observe any significant associations for variants
102 with MAF <1%, suggesting that identified associations were mainly driven by low-frequency,
103 not rare, variants. Among these significant genes, we identified two additional low-frequency
104 missense variants exceeding exome-wide significance for association (*DLEC1,* rs116202356,
105 Glu264Lys, P=2×10^{-10}; *RPL3L*, rs113956264, Val262Met, P=1×10^{-6}; **Table 2**), which were not
106 reported in our single variant tests.
107

108 *eQTL analyses between genes at PWD loci and gene expression*
109 We assessed eQTL associations for top variants and proxies (linkage disequilibrium (LD): r^2>0.8;
110 1000 Genomes: phase 3 version 5, all individuals from LDlink[13]) in two heart tissues from GTEx
111 version 7 (right atrial appendage (RAA) and left ventricle (LV); **Supplemental Table 7**).[14] Six loci
112 were associated with significant changes in gene expression, especially in the RAA, including 2
113 known PWD loci (*HCN1*, *FADS1)* and 4 novel loci (*TTN, TCF21*, *JAZF1*, *SYNPO2L*) **(Supplemental**
114 **Table 7**). The alleles associated with longer PWD at *HCN1* and *SYNPO2L* had lower expression of
115 these genes in RAA tissues. In contrast, alleles at the *JAZF1* and *FADS1* loci were associated with
116 higher gene expression in the RAA and LV, respectively. Gene expression directionality was
117 consistent across RAA and LV tissues. Expression level changes of *JAZF1* and *MYOZ1* per allele in
118 RAA tissue were significantly higher than in the LV. We observed more significant eQTLs in the
119 RAA than the LV, as expected, because P-wave duration reflects atrial conduction.

Relation of the PWD with ECG traits identifies 4 novel and 5 known loci

We examined associations between PWD loci and other ECG measurements from large-scale association studies (**Supplemental Table 8**). We identified 8 novel (*TTN*, *DLEC1*, *ARHGAP10*, *JAZF1*, *SYNPO2L*, *SOX5*, *HMGA2*, *GOSR2*), and 5 known (*SCN10A*, *CAV1*, *FADS1*, *TBX5, MYH6)* PWD loci, all previously reported to be associated with PR interval, PR segment, QRS duration, QT interval, or RR interval. Variants at *TCF21*, *SYNPO2L*, and *MYH6* were associated with PR interval in recent large-scale genetic association studies,[15-17] but the top variants in our PWD analysis were in low to moderate linkage disequilibrium with top variants from these earlier analyses (LD: r^2 <0.8; 1000 Genomes: phase 3 version 5, all individuals) .

Overlap between PWD loci and AF

Fourteen PWD loci were associated with AF risk in a recent AF GWAS[18] (*P* <0.0024=0.05/21 loci; **Figure 1** and **Supplemental Table 8**). Two loci in well-known AF gene regions, *PITX2* and *TTN,* were novel PWD loci. Among these 14 loci, 6 were associated with longer PWD and higher AF risk (*TTN*, *TCF21*, *SOX5*, *GOSR2*, *MC4R*, *MYH6*), whereas 8 were associated with longer PWD but lower AF risk (*DLEC1*, *PITX2*, *CDK6*, *SYNPO2L*, *CAND2*, *SCN10A*, *CAV1*, *TBX5*).

Discussion

In a multi-ancestry study comprising ~65,000 individuals, we identified 12 novel and 7 previously reported loci related to PWD in a meta-analysis of common exome chip variants. After aggregating rare and low-frequency exonic variants, we identified 6 genes, including 2

additional low-frequency variants potentially related to PWD, and loci with specific patterns of association for PWD and AF risk. These findings suggest that AF may result from multiple genetic mechanisms, and PWD may be an endophenotype for these mechanisms.

Our study extends the literature on the genetic components underlying atrial conduction, and the relationship between PWD and AF risk. In comparison to earlier genetic association studies of PWD,[10, 11] we predominantly focused on genetic variants in coding regions (**Table 2**). In total, we identified 21 common variant loci related to PWD. The top common variants explain ~1.6% of the phenotypic variance in PWD. Our gene-based analyses also highlight the importance of low-frequency variants contributing to PWD in genes such as *TTN*, *SCN10A*, and *RPL3L.*

Our findings have two major implications. First, associated loci span genes involved in the development and maintenance of adult cardiac tissue (*PITX2*, *TCF21*, *HMGA2*, *NKX2-5*, *TBX5, CAND2*, *CDK6*), muscle and sarcomere structure (*TTN*, *SYNPO2L*, *SOX5*, *MYH6, RPL3L*), ion channel function (*HCN1, SCN10A*), and cell-cell contact (*PKP1*, *ARHGAP10*, *CAV1*). We additionally noted several genes with a role in metabolism (*JAZF1*, *CDK6*, *HMGA2*, *MC4R*) though the connection to AF is less clear.[19-22] The transcription factor *PITX2* is the top susceptibility locus for AF. Decreased *Pitx2* expression in the adult left atrium is associated with AF in humans,[23] and abnormal cardiac conduction and low-voltage P-waves in knockout mice.[24] *PITX2* is activated by *TBX5* to co-regulate a number of membrane effector genes (such as *SCN5A*, *GJA5* and *RYR2*). Reduction of *Tbx5* expression in a mouse model decreased myocardial automaticity.[25] *TCF21* is a transcription factor required during embryogenesis for formation of heart tissue, and is involved in fibroblast generation after injury in adults.[26] The nuclear

scaffolding protein *HMGA2* trans-activates the heart specific transcription factor *NKX2-5*.[27] *HMGA* overexpression in mice mediates the response to pressure-overload induced cardiac remodeling.[28] *CAND2* suppresses myogenin degradation and directs cardiac progenitor cells towards a myocyte fate.[29]

Titin (*TTN*) is a major structural component of the sarcomere, required for contractile function in cardiomyocytes. Loss of function mutations in *TTN* are associated with early-onset AF[30] and dilated cardiomyopathy.[31] Cytoskeletal Heart-enriched Actin-associated Protein (CHAP, aka *SYNPO2L*), is a Z-disc protein; zebrafish knockdown models display hypertrophy and delayed conduction,[32] and the locus has been associated with AF in GWAS.[18] *SOX5* is a master regulator of cell fate in embryonic development.[33] In drosophila, *SOX5* knockdown results in decreased heart rate and increased cardiac wall thickness.[34] *MYH6*, specifically expressed in the atria, forms the thick filament in cardiac smooth muscle; mutations are associated with cardiomyopathies,[35] sinus node dysfunction,[36] and congenital heart disease.[37] Some identified genes are important for atrial conduction, including *HCN1*[38] and *SCN10A*[39] which govern potassium, and late sodium channel currents, respectively. The proteins *ARHGAP10*,[40] *PKP1*,[41] and *CAV1,*[42] are involved in cell-cell contact and are necessary for efficient signal conduction. The ribosomal protein RPL3L is specifically expressed in skeletal muscle and heart; coding variants in this gene are associated with AF.[43]

Second, our study implicates PWD as a powerful endophenotype for understanding the biological mechanisms of AF. Fifteen loci identified in our study were associated with AF risk in a recent AF GWAS,[18] underscoring the genetic correlation between atrial conduction and AF risk. Epidemiological data indicate that PWD variability is associated with AF risk,[2, 3] AF

recurrence after cardioversion[44] and ablation,[45] as well as ischemic stroke.[46] Generally, we observed that top variants at known sarcomere genes (e.g., *TTN, MYH6*) were associated with increased PWD and increased AF risk, implicating atrial myopathic pathways in AF susceptibility. We speculate that myopathic pathways predispose individuals to AF via delayed conduction velocity, increased propensity for reentry, and susceptibility to ectopic atrial activity. Similarly, *TCF21* and *SOX5* are two transcription factors associated with increased PWD and increased AF risk.

In contrast, top variants at *SCN10A* were associated with increased PWD but reduced AF risk. Other PWD-associated genes, such as *PITX2*, *CAND2, TBX5*, and *CDK6*, contained variants associated with longer PWD and reduced AF risk. The directionality of gene associations observed for PWD and AF risk underscore the complexity of AF susceptibility, while highlighting the potential to leverage PWD to elucidate AF-specific pathways (**Figure 2**). Whether studying PWD can lead to insights relevant for therapeutic targeting remains unclear.

Our results should be interpreted within the context of our study design. First, the majority of our sample consisted of individuals of European ancestry and may have limited generalizability to non-European ancestries. Studies with broader ethnic/racial diversity are warranted. Second, top variants identified in our study may not directly modulate PWD, a limitation of most genetic association studies. Biological characterization of loci is needed to conclusively link variants to function. Third, ascertainment of rare variation is limited using the exome-chip, and future analyses of sequence data are warranted. Fourth, despite a relatively large sample, our findings explained a small proportion of phenotypic variance. Because the additive SNP-based heritability of PWD has been estimated to be as high as 19%,[8] our results

highlight the fact that much of the genetic susceptibility to PWD remains unexplained. Larger samples, genome-wide assessments, and examination of rare variation may be necessary to identify additional loci for PWD.

In conclusion, we identified 14 novel loci in common and low-frequency variant analyses and 6 gene regions in a low-frequency variant analysis for PWD. Our findings highlight the shared genetic components of atrial conduction and AF risk, and illustrate the diverse biological pathways affecting atrial conduction and mechanisms leading to AF.

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403 **Tables**

404 **Table 1. Study participant characteristics***

Study	Ancestry	N	Age, years, mean±SD	Sex, women, %	P-wave duration, milliseconds, mean±SD	RR interval, milliseconds, mean±SD
ARIC	European	8861	53.9±5.7	54.1	106.0±11.8	920.5±133.8
	African	2922	53.3±5.8	62.2	111.5±11.9	924.2±148.6
BRIGHT	European	195	60.5±8.9	57.4	121.1±19.4	976.1±186.0
CAMP	European	1887	59.9±10.4	37.4	106.0±15.8	936.8±171.3
CHS	European	2648	72.3±5.4	60.7	109.9±13.0	950.0±145.8
	African	445	72.6±5.6	64.5	112.2±13.1	912.8±156.4
ERF	European	514	49.0±14.3	54.1	111.2±12.4	963.4±152.9
FHS	European	5677	47.2±13.3	55.0	105.0±12.0	973.7±155.9
INTER99	European	5872	46.2±7.9	51.6	104.3±12.5	920.4±150.5
KORA	European	2435	47.1±12.8	51.9	108.0±11.1	939.7±147.7

LIFELINES	European	1914	45.2±13.0	59.8	112.1±12.4	897.3±144.5
UHP	European	1657	38.5±12.5	55.8	109.1±14.6	956.5±152.4
MESA	European	2083	61.8±10.1	51.8	104.4±12.9	1054.5±158.9
	African	1131	61.3±10.3	52.9	107.9±12.3	1054.4±170.2
	Hispanic	1186	60.6±10.3	50.1	105.2±12.0	1061.0±154.5
	Asian	630	61.3±10.3	50.2	101.7±11.7	1059.0±140.3
NEO	European	5119	55.6±6.0	51.9	114.2±13.9	933.8±150.5
RS	European	1740	69.5±8.4	51.4	120.1±12.4	859.8±140.6
SHIP-0	European	2653	46.5±15.4	51.8	109.5±11.2	853.6±147.8
SHIP-Trend	European	2922	47.9±14.6	52.5	113.1±11.9	911.3±134.5
WHI	European	10766	65.8±6.6	100	107.2±11.9	914.3±134.2
	African	1183	64.3±6.5	100	110.6±11.5	920.2±143.7

405 *N: sample size

406

Table 2. Top exome-wide significant variants for P-wave duration in multi-ethnic meta-analysis*

								Residuals					Inverse normal transformed residuals				
Locus	**Closest gene**	**Location**	**rsID**	**EA**	**Function**	**N**	**EAF**	**Beta**	**SE**	***P***	**h^2(%)**	**I^2(%)**	**Beta**	**SE**	***P***	**h^2(%)**	**I^2(%)**
Novel loci																	
1	*PKP1*	1q32.1	rs1626370	A	missense	64431	0.2	0.39	0.08	**2×10^{-6}**	0.04	2	0.03	0.01	**2×10^{-6}**	0.04	0
2	*TTN*†	2q31.2	rs2042995	C	intron	64410	0.3	0.41	0.08	**4×10^{-7}**	0.04	8	0.03	0.01	**5×10^{-7}**	0.04	12
3	*DLEC1*‡	3p22.2	rs116202356	G	missense	64331	0.98	1.72	0.27	**2×10^{-10}**	0.06	20	0.14	0.02	**2×10^{-10}**	0.06	19
4	*PITX2*	4q25	rs17042171	C	intergenic	64399	0.9	0.64	0.10	**8×10^{-11}**	0.07	45	0.06	0.01	**2×10^{-11}**	0.07	50
5	*ARHGAP10*	4q31.23	rs6845865	C	intron	64437	0.2	0.54	0.09	**2×10^{-10}**	0.06	0	0.05	0.01	**9×10^{-11}**	0.07	0
6	*TCF21/TARID*	6q23.2	rs2327429	C	upstream	64434	0.3	0.39	0.07	**2×10^{-7}**	0.04	13	0.03	0.01	**1×10^{-7}**	0.04	9
7	*JAZF1*	7p15.1	rs864745	C	intron	64388	0.5	0.32	0.07	2×10^{-6}	0.04	0	0.03	0.01	**1×10^{-6}**	0.04	0
8	*CDK6*	7q21.2	rs2282978	C	intron	64424	0.4	0.39	0.07	**2×10^{-8}**	0.05	0	0.03	0.01	**5×10^{-8}**	0.05	6
9	*SYNPO2L*	10q22.2	rs3812629	A	missense	64423	0.2	0.47	0.09	**4×10^{-7}**	0.04	0	0.04	0.01	**7×10^{-7}**	0.04	0

10	*SOX5*	12p12.1	rs17287293	A	intergenic	64429	0.9	0.49	0.10	**3×10^{-7}**	0.04	0	0.04	0.01	**3×10^{-7}**	0.04	0
11	*HMGA2*	12q14.3	rs8756	C	3‘-UTR	64418	0.5	0.33	0.07	**7×10^{-7}**	0.04	0	0.03	0.01	**5×10^{-7}**	0.04	0
12	*RPL3L*‡	16p13.3	rs113956264	C	missense	64403	0.97	0.99	0.20	**1×10^{-6}**	0.04	0	0.08	0.02	4×10^{-6}	0.03	10
13	*GOSR2*	17q21.32	rs17608766	C	intron	64435	0.1	0.80	0.10	**9×10^{-15}**	0.09	0	0.07	0.01	**1×10^{-15}**	0.10	0
14	*MC4R*	18q21.32	rs12970134	A	intergenic	64430	0.3	0.38	0.08	**1×10^{-6}**	0.04	0	0.03	0.01	**7×10^{-6}**	0.03	0
Previously reported loci																	
15	*CAND2*	3p25.2	rs11718898	T	missense	52472	0.3	0.39	0.08	**9×10^{-7}**	0.05	0	0.03	0.01	8×10^{-7}	0.05	0
	CAND2	3p25.2	rs3732675	T	missense	64395	0.4	0.34	0.07	1×10^{-6}	0.04	0	0.03	0.01	**3×10^{-7}**	0.04	0
16	*SCN10A*	3p22.2	rs6800541	C	intron	64423	0.4	1.18	0.07	**4×10^{-63}**	0.44	51	0.10	0.01	**2×10^{-65}**	0.45	45
17	*HCN1*	5p12	rs6892594	T	intron	64427	0.4	0.43	0.07	**2×10^{-10}**	0.06	0	0.04	0.01	**3×10^{-10}**	0.06	0
18	*CAV1*	7q31.2	rs3807989	A	intron	64430	0.4	0.47	0.07	**2×10^{-12}**	0.08	0	0.04	0.01	**8×10^{-13}**	0.08	0
19	*FADS1*	11q12.2	rs174546	C	3’-UTR	64430	0.7	0.50	0.07	**2×10^{-11}**	0.07	9	0.04	0.01	**6×10^{-12}**	0.07	9
20	*TBX5*	12q24.21	rs883079	C	3’-UTR	64435	0.3	0.80	0.07	**9×10^{-28}**	0.19	17	0.07	0.01	**6×10^{-29}**	0.19	11
21	*MYH6*	14q11.2	rs452036	A	intron	64422	0.4	0.68	0.07	**8×10^{-23}**	0.15	0	0.06	0.01	**1×10^{-23}**	0.16	0

*EA: effect allele, N: sample size, EAF: effect allele frequency, Beta: the changes of (inverse normal transformed) P-wave duration residuals per 1 effect allele increment, SE: standard error, h^2: SNP heritability estimate. *P*-values in bold are at exome-wide significance.

†Locus with minor allele frequency <5% is also identified from gene-based analysis

‡Locus with minor allele frequency <5% identified from gene-based analysis

413 **Table 3. Top gene in low frequency variant gene-based analyses of P-wave duration stratified by ancestral group.**

	Multi-ethnic				European				African			
				Inverse normal transformed				Inverse normal transformed				Inverse normal transformed
	Var		Residuals	residuals	Var		Residuals	residuals	Var		Residuals	residuals
Gene	**#**	**cMAC**	***P*†**	***P***	**#**	**cMAC**	***P***	***P***	**#**	**cMAC**	***P***	***P***
SKAT												
TTN	775	276986	**5×10^{-27}**	**5×10^{-26}**	704	215801	**5×10^{-27}**	**1×10^{-26}**	536	23041	0.59	0.71
DLEC1	57	10419	**2×10^{-13}**	**2×10^{-13}**	55	6937	**2×10^{-12}**	**3×10^{-12}**	39	2568	0.70	0.73
TTC21A	37	12207	1×10^{-5}	5×10^{-6}	32	10900	4×10^{-6}	**1×10^{-6}**	28	1250	0.98	0.98
SCN10A	61	16550	**7×10^{-8}**	**9×10^{-9}**	47	12804	**2×10^{-7}**	**4×10^{-8}**	34	524	0.84	0.81
RPL3L	26	8510	**1×10^{-6}**	4×10^{-6}	25	6742	**2×10^{-6}**	1×10^{-5}	18	265	0.33	0.21
Burden												
TTN	775	276986	**1×10^{-14}**	**8×10^{-14}**	704	215801	**1×10^{-20}**	**4×10^{-18}**	536	23041	0.26	0.27

MUC5B	68	36414	**7×10^{-6}**	1×10^{-5}	63	25110	**3×10^{-6}**	6×10^{-6}	58	2846	0.59	0.56

Var#: number of variants included in the gene set, cMAC: cumulative minor allele count.

P-values in bold exceed the exome-wide significance threshold (P-value <3.0×10^{-6}, 3.1×10^{-6}, and 3.5×10^{-6} for individuals of multi-ethnic, European, and African ancestries, respectively).

Figure legends

Figure 1. P-wave duration loci and atrial fibrillation risk. The x-axis represents the association between the top P-wave duration (PWD) loci and PWD in -$\log_{10}$ scale. The y-axis represents the association *P*-value between the top PWD loci and atrial fibrillation (AF) risk (-$\log_{10}$ scale). Variants above y=0 refer to loci associated with longer PWD and higher AF risk (colored in yellow). Variants below y=0 refer to loci associated with longer PWD but lower AF risk (colored in blue). Displayed results are from the multi-ethnic meta-analysis of PWD residuals. Associations with AF were derived from a recent AF GWAS.[18] Dashed lines show the significance threshold for the current exome-wide analysis (vertical; *P*-value<1.9×10^{-6}) and for prior genome-wide analyses of AF (horizontal; *P*-value<5×10^{-8}). The dotted line represents the significance cutoff after Bonferroni correction (horizontal; *P*-value<2.4×10^{-3}=0.05/21 PWD loci).

Figure 2: Identified P-wave duration associated genes highlight multiple biological pathways for atrial fibrillation risk. Gene with *increasing* risk of AF coupled with prolonged PWD are listed at the right. Gene with *decreasing* risk of AF coupled with prolonged PWD are listed at the left. Each gene is accompanied by a diagram representing the biological function of the gene, indicating how the gene may affect PWD.

439 **Figures**

440 **Figure 1. P-wave duration loci and atrial fibrillation risk**

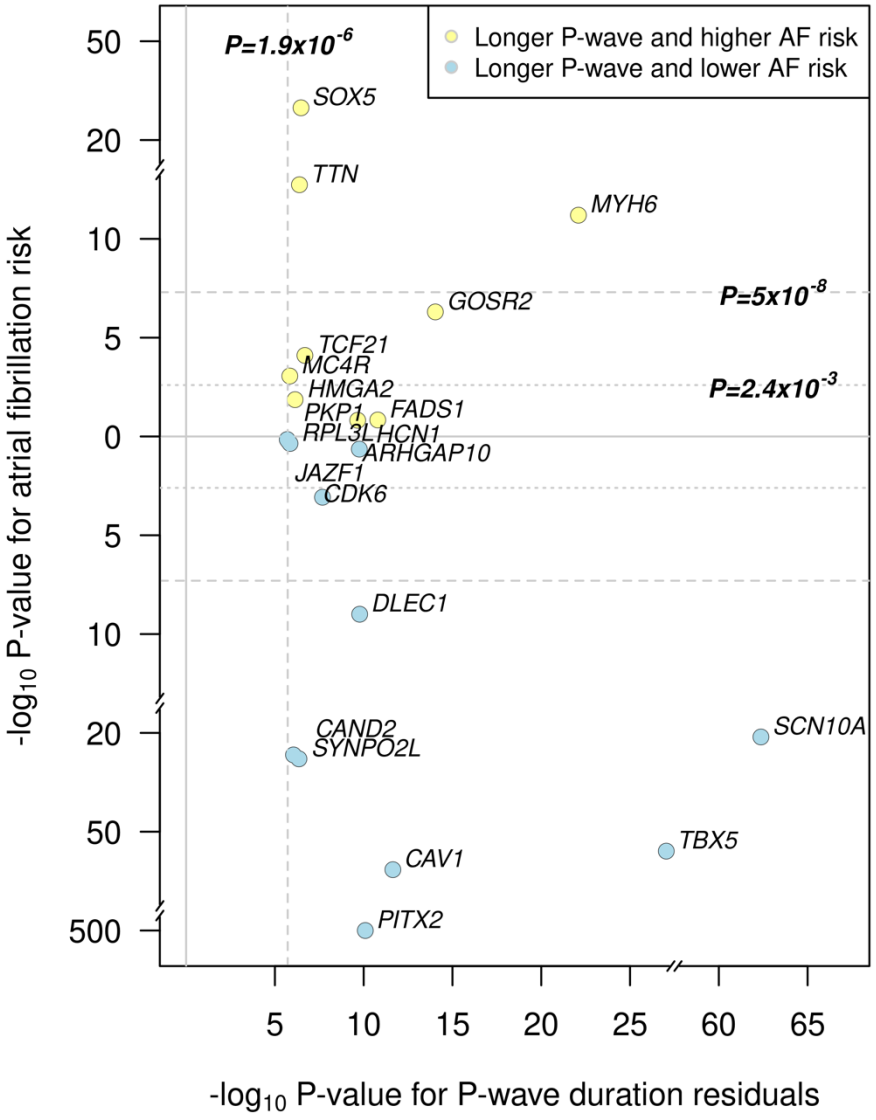

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Figure 2: Identified P-wave duration associated genes highlight multiple biological pathways for atrial fibrillation risk