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A Missense Variant in *PLEC* Increases Risk of Atrial Fibrillation

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ABSTRACT

BACKGROUND Genome-wide association studies (GWAS) have yielded variants at >30 loci that associate with atrial fibrillation (AF), including rare coding mutations in the sarcomere genes *MYH6* and *MYL4*.

OBJECTIVES The aim of this study was to search for novel AF associations and in doing so gain insights into the mechanisms whereby variants affect AF risk, using electrocardiogram (ECG) measurements.

METHODS The authors performed a GWAS of 14,255 AF cases and 374,939 controls, using whole-genome sequence data from the Icelandic population, and tested novel signals in 2,002 non-Icelandic cases and 12,324 controls. They then tested the AF variants for effect on cardiac electrical function by using measurements in 289,297 ECGs from 62,974 individuals.

RESULTS The authors discovered 2 novel AF variants, the intergenic variant rs72700114, between the genes *LINC01142* and *METTL11B* (risk allele frequency = 8.1%; odds ratio [OR]: 1.26; $p = 3.1 \times 10^{-18}$), and the missense variant p.Gly4098Ser in *PLEC* (frequency = 1.2%; OR: 1.55; $p = 8.0 \times 10^{-10}$), encoding plectin, a cytoskeletal cross-linking protein that contributes to integrity of cardiac tissue. The authors also confirmed 29 reported variants. p.Gly4098Ser in *PLEC* significantly affects various ECG measurements in the absence of AF. Other AF variants have diverse effects on the conduction system, ranging from none to extensive.

CONCLUSIONS The discovery of a missense variant in *PLEC* affecting AF combined with recent discoveries of variants in the sarcomere genes *MYH6* and *MYL4* points to an important role of myocardial structure in the pathogenesis of the disease. The diverse associations between AF variants and ECG measurements suggest fundamentally different categories of mechanisms contributing to the development of AF. (J Am Coll Cardiol 2017;70:2157–68)

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ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

AVN = atrioventricular node

EBS = epidermolysis bullosa simplex

ECG = electrocardiogram

LD = linkage disequilibrium

SNP = single nucleotide polymorphism

SSS = sick sinus syndrome

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in humans and a significant cause of morbidity and mortality (1-3). More than 30 rare sequence variants with large effects have been identified in familial AF, including coding variants in ion channels, signaling molecules, structural proteins, and transcription factors (4). These mutations explain only a small proportion of AF cases but can provide important insights into the mechanisms of AF. For example, mutations in the potassium channel gene *KCNQ1*, the first gene linked to familial AF, are thought to cause AF by reducing the atrial action potential duration and the effective refractory period (5). Over the last decade, genome-wide association studies (GWAS) have yielded variants at >30 loci that associate with nonfamilial AF as a primary or secondary trait, with the most notable association being at *PITX2*, a transcription factor gene that affects cardiac development (6). However, most of the AF variants discovered through GWAS are in noncoding regions, and the mechanisms by which they affect AF remains to be elucidated (6-19). Recently, GWAS in Iceland based on whole-genome sequencing have associated coding variants in the sarcomere genes *MYH6* and *MYL4* with AF (13,14).

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The use of electrocardiogram (ECG) measurements as intermediate traits of arrhythmia has proven valuable and has led to the discovery of variants that affect both the cardiac electrical function and the risk of arrhythmia, including variants that associate with both the PR interval and risk of AF (15-17). Assessing the effects of both coding and noncoding AF variants on detailed ECG traits could give valuable information about their modes of action and the pathophysiology of AF.

Here we describe a GWAS of AF in 14,255 Icelandic cases and 374,939 controls based on whole-genome sequencing and testing of the AF variants for association with closely related phenotypes including sick sinus syndrome (SSS) and ischemic stroke. We then tested the resulting AF variants and those previously described for associations with ECG traits.

METHODS

Detailed methods are available in the Online Appendix. The study was approved by the Icelandic Data Protection Authority and the National Bioethics Committee of Iceland. The study complies with tenets of the Declaration of Helsinki.

STUDY POPULATIONS. The Icelandic AF population consisted of all patients with the diagnosis of AF (International Classification of Diseases-10 [ICD-10] code I.48 and ICD-9 code 427.3) at Landspitali, the National University Hospital, in Reykjavik and Akureyri Hospital (the 2 largest hospitals in Iceland) from 1987 to 2015. All AF cases (N = 14,255) were included. Controls consisted of 374,939 Icelanders recruited through different genetic research projects at deCODE genetics (Reykjavik, Iceland). Individuals in the AF cohort were excluded from the control group (see Online Table 1 for subject and control characteristics). We followed up the novel AF variants in AF sample sets from the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) trial (20) (1,238 cases and 11,562 controls) and the Vanderbilt AF Registry (764 cases and 762 controls) (Online Methods). The deCODE genetics phenotype database contains extensive medical information on various diseases and traits. This includes sample sets from a pacemaker population (n = 3,578), SSS subjects (n = 3,310), ischemic stroke subjects (n = 5,626), and cardioembolic stroke subjects (n = 1,369). The controls used in the various case control analyses of this study consisted of individuals randomly drawn from the Icelandic genealogical database and individuals from other genetic studies at deCODE genetics.

ELECTROCARDIOGRAM DATA. ECG data were collected from Landspitali, the National University Hospital, in Reykjavik and included all ECGs obtained and digitally stored from 1998 to 2015, a total of 434,000 ECGs from 88,217 individuals. These were ECGs obtained from all hospital departments from both in- and outpatients. For the main analysis, sinus rhythm (heart rate 50 to 100 beats/min) ECGs from individuals without the diagnosis of AF were used. This was done to assess the effect of the AF variants on ECG measurements (e.g., P-wave morphology, PR intervals, and so forth) and thus cardiac electrical function in the absence of AF. Individuals with pacemakers also were excluded. This resulted in 289,297 sinus rhythm ECGs from 62,974 individuals. The analysis was also done using all ECGs regardless of rhythm and history of AF (Online Appendix).

GENERATION OF GENOTYPE DATA. This study was based on whole-genome sequence data from 15,220 Icelanders participating in various disease projects at deCODE genetics. The sequencing was done using standard TruSeq methodology (Illumina, San Diego, California) to a mean depth of 35 ± 8 x, as previously described (21). Genotypes of the 32.5 million sequence variants identified through sequencing (single nucleotide polymorphisms [SNPs] and indels) were

TABLE 1 Variants Associating Genome-Wide Significantly With AF (N = 13,471)

Reference SNP ID	Chr	Pos (hg38)	Closest Gene(s)	Risk Allele/ Other	RAF, %	Coding Effect	OR (95% CI)	p Value	p Value Threshold	Reported SNP ID (Ref. #)	r^2 With Reported SNP
Novel signals											
rs72700114	1q24	170,224,684	*METTL11B*, *LINC01142*	C/G	8.1	Intergenic	1.22 (1.15–1.29)	7.0×10^{-12}	2.3×10^{-9}	NA	NA
rs373243633	8q24	143,917,940	*PLEC*	T/C	1.2	Missense	1.55 (1.35–1.78)	8.0×10^{-10}	5.1×10^{-8}	NA	NA
Variants reported to associate with AF											
rs6843082	4q25	110,796,911	*PITX2*	G/A	20.1	Intergenic	1.43 (1.38–1.49)	1.6×10^{-73}	2.3×10^{-9}	rs6843082 (10)	1.00
rs387906656*	14q11	23,396,970	*MYH6*	A/G	0.3	Missense	2.43 (1.94–3.04)	1.1×10^{-14}	5.1×10^{-8}	rs387906656 (13)	1.00
rs34245846	1q21	154,858,667	*KCNN3*	G/A	32.7	Intronic	1.15 (1.11–1.19)	1.2×10^{-14}	7.9×10^{-10}	rs13376333 (10)	0.52
rs2359171	16q22	73,019,123	*ZFHX3*	A/T	18.8	Intronic	1.17 (1.13–1.22)	1.5×10^{-14}	2.3×10^{-9}	rs2106261 (8)	1.00
rs148321568	10q22	73,668,538	*SYNPO2L*, *MYOZ1*	A/T	85.2	Intergenic	1.19 (1.14–1.25)	3.7×10^{-13}	7.9×10^{-10}	rs10824026 (9)	0.95
rs11598047	10q24	103,582,915	*NEURL1*	G/A	17.9	Intronic	1.15 (1.10–1.19)	1.5×10^{-10}	2.3×10^{-9}	rs12415501 (11)	0.91
rs651386	1q24	170,622,169	*PRRX1*	A/T	57.4	Intergenic	1.11 (1.08–1.15)	1.9×10^{-10}	7.9×10^{-10}	rs3903239 (9)	0.61
rs1572226	6q22	118,313,916	*SLC35F1*, *PLN*	C/G	42.4	Intronic	1.11 (1.08–1.15)	3.1×10^{-10}	2.3×10^{-9}	rs4946333 (18)	0.56

*Locus previously associating with AF but not genome-wide significantly.

AF = atrial fibrillation; Chr = chromosome; CI = confidence interval; OR = odds ratio; NA = not applicable; Pos = position; RAF = risk allele frequency; SNP = single nucleotid polymorphism.

then imputed into 151,677 Icelanders chip typed using SNP chips (Illumina) and their close relatives (familial imputation). Imputation refers to the statistical inference of unobserved genotypes using known haplotypes in the population (see Online Methods for further details).

STATISTICAL ANALYSIS. Logistic regression was used to test for association between SNPs and AF and other traits, treating disease status as the response and allele counts from direct genotyping or expected genotype counts from imputation as covariates. To account for inflation in test statistics due to cryptic relatedness and stratification, we applied the method of linkage disequilibrium (LD) score regression (22). The estimated correction factor for AF based on LD score regression was 1.38 for the additive model. The threshold for genome-wide significance was corrected for multiple testing with a weighted Bonferroni adjustment, using as weights the enrichment of variant classes with predicted functional impact among association signals (see Online Methods for significance thresholds for specific groups of variants) (23). We tested 31 AF variants for association with 122 ECG measurements (Online Table 2), using linear regression, treating the ECG measurement as the response and the genotype as the covariate. The ECG measurements were adjusted for sex, year of birth, and age at measurement and were subsequently standardized to have a normal distribution. For individuals with multiple ECG measurements, the mean standardized value was used. The Benjamini-Hochberg false discovery rate procedure controlling the false discovery rate at 0.05 at each marker was used to account for multiple testing.

RESULTS

We tested ~32.5 million sequence variants for association with AF in 14,255 Icelandic cases and 374,939 controls. Variants were identified by whole-genome sequencing of 15,220 Icelanders and imputed into 151,677 long-range phased individuals and their relatives (21). We found 2 novel AF associations, an intergenic variant at the genes *METTL11B* and *LINC01142*, and a missense variant in the gene *PLEC*. We also replicated 29 loci previously associated with AF in GWAS, either as a primary or secondary trait, following association with SSS, heart rate, or PR interval (**Tables 1 and 2,** Online Figure 1). Variants at 10 loci reached genome-wide significance (**Table 1**). One of those had only been previously associated with AF as a secondary trait, a missense variant in *MYH6* reported in SSS (13) (see Online Figure 2 for loci plots). Two of the previously reported variants (rs12044963 at *KCND3* and rs2047036 at *SH3PXD2A*) were previously reported in Japanese subjects and nominally associated with AF in Europeans (19). Here we replicated the previously reported variants and provide further evidence that these are true AF variants. Additional variants reported in Japanese subjects were either not replicated (**Table 2**) or are very rare or nonexistent in the Icelandic population (Online Table 3).

A MISSENSE VARIANT IN *PLEC* INCREASES RISK OF AF. The novel AF association with *PLEC* on chromosome 8 is driven by a low-frequency missense variant p.Gly4098Ser (NP_958782.1; frequency = 1.2%) that associates with AF with an odds ratio (OR) of 1.55

TABLE 2 Replication of Loci Previously Reported to Associate With AF (N = 13,471)

Ref SNP (ID) (Ref. #)	Chr	Pos (hg38)	Closest Gene(s)	Risk Allele/ Other	RAF, %	Coding Effect	OR (95% CI)	p Value*
Variants reported to associate genome-wide significantly with AF								
rs3807989 (9)	7q31	116,546,187	*CAV1*	G/A	59.5	Intronic	1.11 (1.07–1.14)	**2.5×10^{-9}**
rs10821415 (9)	9q22	94,951,177	*C9orf3*	A/C	38.5	Intronic	1.10 (1.06–1.14)	**3.8×10^{-8}**
rs35176054 (18)	10q24	103,720,629	*SH3PXD2A*	A/T	13.6	Intronic	1.14 (1.08–1.19)	**9.3×10^{-8}**
rs72700118 (18)	1q24	170,225,682	*METTL11B, LINC01142*	A/C	13.4	Intergenic	1.12 (1.07–1.17)	**$2,9 \times 10^{-6}$**
rs10507248 (11)	12q24	114,359,288	*TBX5*	T/G	70.2	Intronic	1.09 (1.05–1.13)	**3.0×10^{-6}**
rs7164883 (9)	15q24	73,359,833	*HCN4*	G/A	14.7	Intronic	1.12 (1.07–1.17)	**1.1×10^{-6}**
rs7508 (18)	8p22	18,056,461	*ASAH1*	A/G	71.5	3′ UTR	1.09 (1.05–1.13)	**1.2×10^{-5}**
rs1152591 (9)	14q23	64,214,130	*SYNE2*	A/G	51.0	Upstream	1.07 (1.04–1.11)	**3.5×10^{-5}**
rs13216675 (11)	6q22	122,131,183	*GJA1*	T/C	66.2	Intergenic	1.08 (1.04–1.11)	**3.5×10^{-5}**
rs11047543 (18)	12p12	24,635,405	*SOX5*	G/A	86.7	Intergenic	1.11 (1.05–1.16)	**5.3×10^{-5}**
rs2540949 (18)	2p14	65,057,097	*CEP68*	A/T	59.3	Intronic	1.07 (1.03–1.10)	**1.5×10^{-4}**
rs12044963 (19)	1p13	111,849,738	*KCND3*	T/G	9.4	Intronic	1.11 (1.05–1.17)	**2.1×10^{-4}**
rs4642101 (11)	3p25	12,800,724	*CAND2*	G/T	65.8	Intronic	1.07 (1.03–1.10)	**3.1×10^{-4}**
rs6800541 (18)	3p22	38,733,341	*SCN10A*	T/C	63.6	Intronic	1.06 (1.03–1.10)	**4.3×10^{-4}**
rs3771537 (18)	2p13	69,811,660	*ANXA4*	A/C	53.1	Intronic	1.06 (1.03–1.10)	**4.6×10^{-4}**
rs75190942 (18)	11q24	128,894,676	*KCNJ5*	A/C	10.6	Downstream	1.09 (1.03–1.15)	**0.0016**
rs337711 (18)	5q22	114,412,874	*KCNN2*	T/C	34.1	Intronic	1.05 (1.01–1.08)	**0.0071**
rs2047036 (19)	10q24	103,717,405	*SH3PXD2A*	C/T	40.1	Intronic	1.04 (1.01–1.08)	**0.012**
rs2288327 (18)	2q31	178,546,938	*TTN*	G/A	14.0	Intronic	1.06 (1.01–1.11)	**0.017**
rs2967791 (18)	5q31	137,677,417	*KLHL3*	T/C	56.7	Intronic	1.04 (1.00–1.07)	**0.025**
rs7698692 (19)	4q34	173,682,953	*HAND2*	G/A	2.1	Intergenic	1.10 (0.99–1.23)	0.089
rs17461925 (19)	1q32	203,057,463	*PPFIA4*	A/G	66.2	Intronic	1.01 (0.97–1.04)	0.69
rs6490029 (11)	12q24	111,260,653	*CUX2*	A/G	29.8	Intronic	1.00 (0.98–1.02)	1.00
Variants reported to associate with AF as a secondary trait, following association with heart rate and/or PR interval								
rs6882776 (15)	5q35	173,237,160	*NKX2-5*	G/A	71.8	Upstream	1.09 (1.05–1.13)	**8.1×10^{-6}**
rs11708996 (16)	3p22	38,592,432	*SCN5A*	G/C	88.2	Intronic	1.04 (0.99–1.09)	0.14

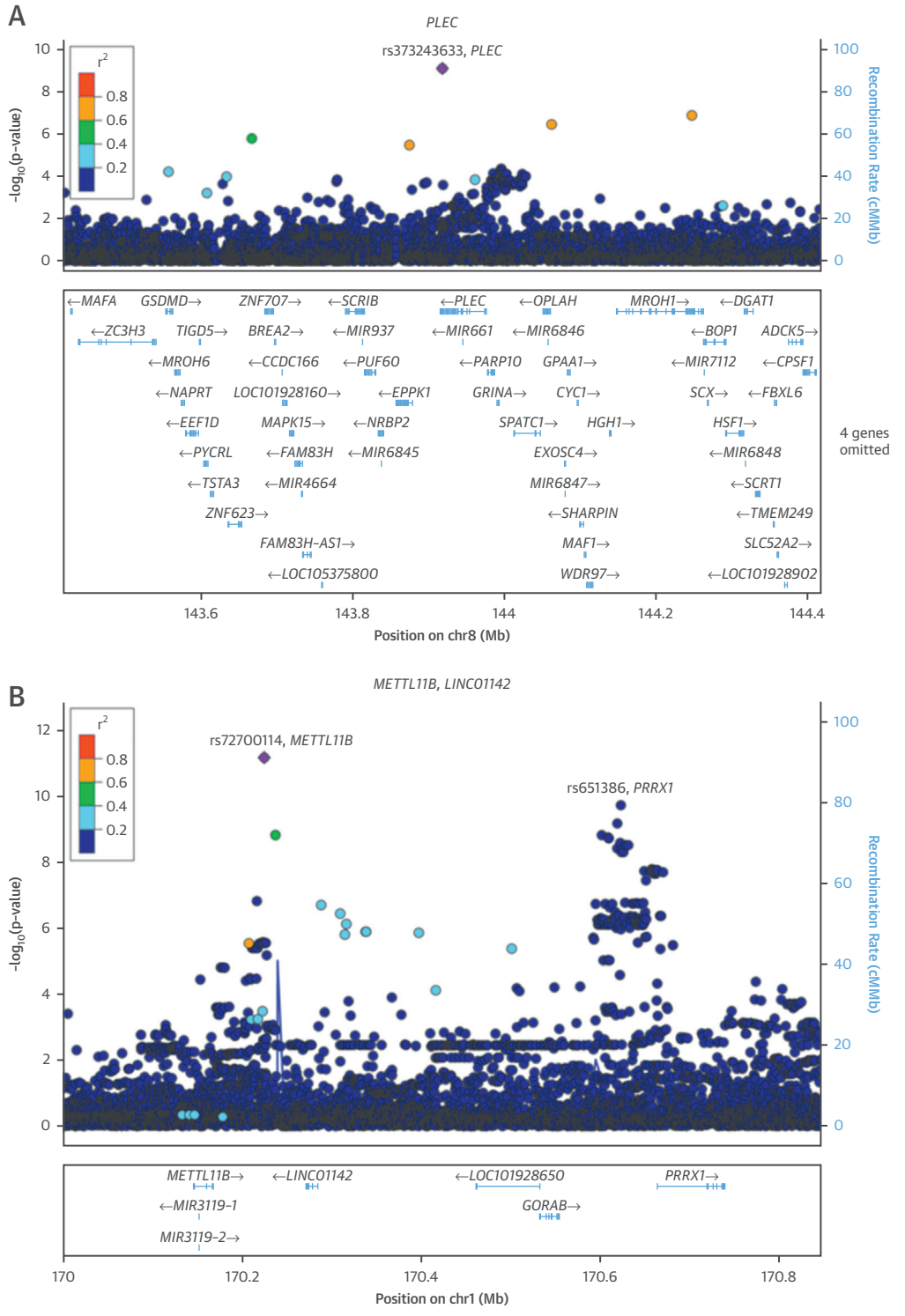
*Significant p values (<0.05) are in **bold**.
Abbreviations as in Table 1.

(95% confidence interval [CI]: 1.35 to 1.78; $p = 8.0 \times 10^{-10}$); (**Figure 1A**). It is the only variant in the region reaching genome-wide significance. Conditional analysis did not reveal additional signals in the region, and no other coding variant in *PLEC* associates independently with AF (Online Table 4). *PLEC* encodes plectin, a very large (>500 kDa) multidomain cytoskeletal linking protein with a role in maintaining tissue integrity in skin, striated muscle, and heart (24). It is expressed in many tissues, including the heart, with equal expression in atria and ventricles (25).

p.Gly4098Ser is a missense variant (C→T at position 143,917,940) in exon 32/32 of *PLEC* and is potentially damaging (Polymorphism Phenotyping v2 [PolyPhen-2]: 1; Sorting Intolerant From Tolerant [SIFT]: 0.02) (26,27). Generally, the *PLEC* gene is tolerant to missense mutations (constrained metric z-score: −0.38 [28]). However the region in question is conserved and has low tolerance to mutations according to Genomic Evolutionary Rate Profiling (GERP) score (4.2 [29]) and sub-region Residual Variation Intolerance Score (subRVIS) (<35th percentile [30]). Exon 32 encodes the C-terminal intermediate filament binding domain of plectin, and the variant results in a glycine to serine substitution on the surface, close to a suggested interaction site for intermediate filaments (Online Figure 3) (31).

The carrier frequency of p.Gly4098Ser among genotyped individuals was 3.08% (269 of 8,740 subjects) in cases and 2.09% (2,752 of 131,941 subjects) in controls. Seventeen of the 151,677 genotyped study participants (currently at a mean 46 years of age [range: 11 to 80 years]) were homozygous for p.Gly4098Ser. No homozygote had been diagnosed with AF according to our records. P.Gly4098Ser did not associate with AF ($p = 0.37$) or ECG traits (lowest p value = 0.0069 in T amplitude lead V_3; beta = −1.25) under a recessive mode of inheritance. The fact that none of the 17 homozygotes had been diagnosed with AF was unexpected but does not constitute a significant deviation from the additive model ($p = 0.093$). Furthermore, it is possible that some of them have AF that has not been diagnosed or who may develop AF later in life.

FIGURE 1 **Regional Plots of Novel AF Loci**



Regional plots of **(A)** the *PLEC* locus on chromosome 8q24 and **(B)** a signal, rs72700114, between *METTL11B* and *LINC01142* on chromosome 1q24, and a previously reported signal 400 kb away, rs651386, close to *PRRX1*. The plots depict association results (p values) with atrial fibrillation (N = 13,471). For each plot, the y-axis shows the $-\log_{10}$ p values, and the x-axis shows the genomic position (hg38). The leading variants of independent signals are labeled as **diamonds** and are **purple**. Other variants are colored according to correlation (r^2) with the leading marker.

The p.Gly4098Ser mutation is exceedingly rare outside Iceland. The variant was only found in 13 of the 140,842 individuals in the Genome Aggregation Database (12 Europeans, 1 South Asian) (28). The Icelandic population is a founder population, in that a small number of ancestors accounts for a relatively large proportion of the population. Hence, variants that are very rare in more outbred populations, like p.Gly4098Ser, may be more common in Iceland. Acknowledging the limited power to detect associations, we genotyped the variant in 2 AF sample sets, from the Vanderbilt AF registry (764 cases and 762 controls) and the FOURIER trial (1,238 cases and 11,562 controls). We found 3 carriers, and all had AF.

The second new AF variant, rs72700114 on chromosome 1q24 (risk allele frequency = 8.1%), is located between the genes *LINC01142* and *METTL11B* and is associated with AF with an OR of 1.22 ($p = 7.0 \times 10^{-12}$; 95% CI: 1.15 to 1.29) in the Icelandic GWAS. The association replicated well in the non-Icelandic AF sample sets and the combined OR was 1.26 (95% CI: 1.19 to 1.32; p value of 3.1×10^{-18}) (Online Table 5). Variant rs72700114 is located 400 kb from a previously reported AF locus, represented by rs651386, close to *PRRX1* (**Figure 1B**) (9). The 2 variants are not strongly correlated ($D' = 0.38$; $r^2 = 0.009$), and when conditioned on each other, they both associate with AF (Online Table 6). In fact, rs72700114 is the second variant to be associated with AF secondarily with this locus. The recently reported variant rs72700118 (18) is located only 1 kb away from rs72700114, but the 2 variants are not in LD ($D' = 1$; $r^2 = 0.014$). None of the 3 variants associates with the expression of nearby genes in our samples from blood (2,528 samples) or adipose tissue (686 samples).

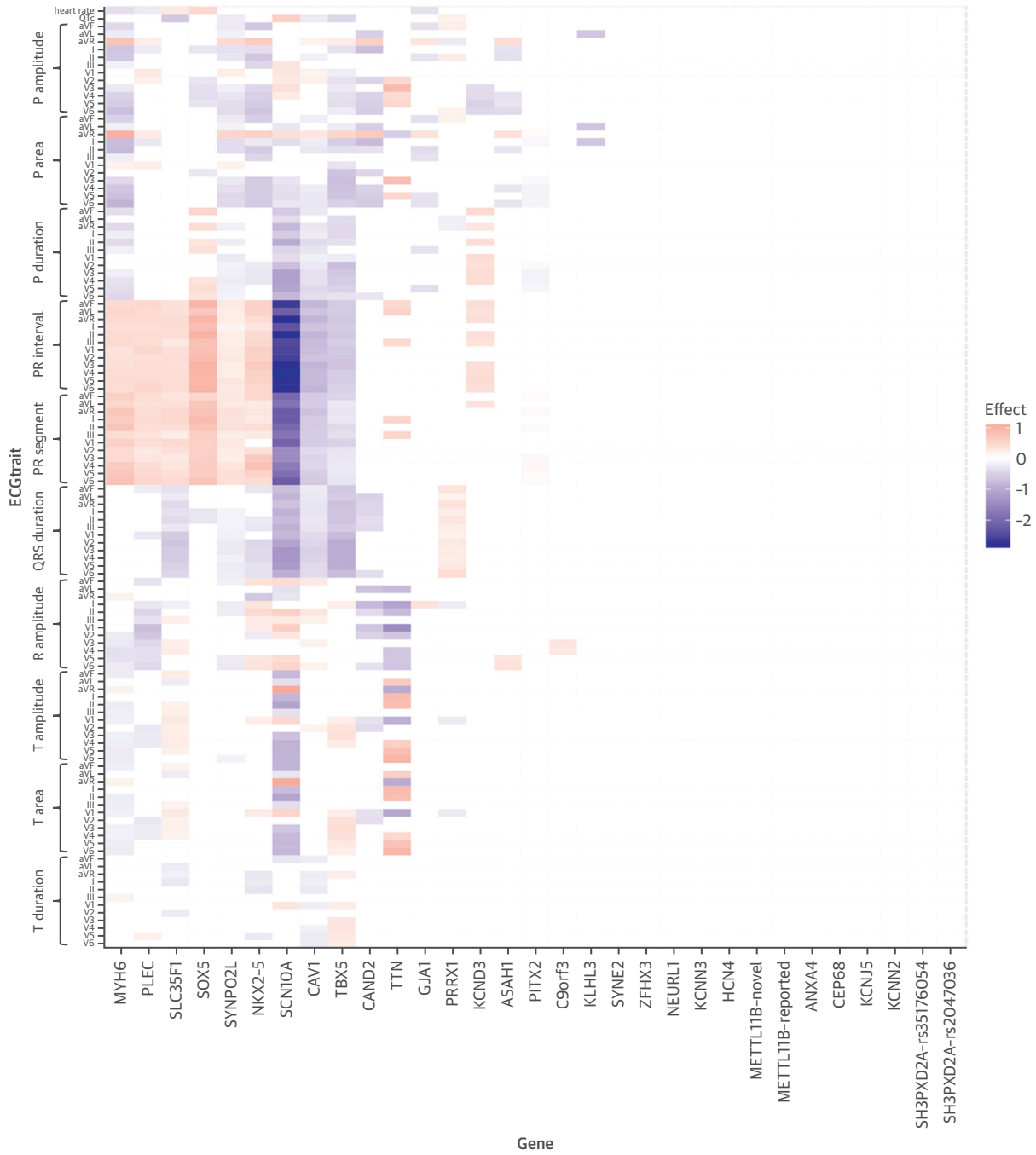
ASSOCIATION OF AF VARIANTS WITH ECG MEASUREMENTS IN THE ABSENCE OF AF. We tested the 2 novel and 29 previously reported AF variants for association with ECG traits that reflect electrophysiological functions of the heart in sinus rhythm (**Figure 2,** Online Table 7, Online Figure 4). A total of 289,297 sinus rhythm ECGs from 62,974 individuals without the diagnosis of AF were included in the analysis. We tested all variants for association with 122 ECG variables, some of them correlated (Online Table 2). Independent of AF diagnoses, the novel p.Gly4098Ser variant in *PLEC* associates with many ECG measurements. The variant affects P-wave amplitude and area, prolongs the PR segment representing atrioventricular node (AVN) conduction, and lowers the R wave amplitude. The coding AF variant in *MYH6*, another structural gene, also associated with SSS and a 6-fold risk of pacemaker placement, has a similar general effect on the cardiac electrical function, albeit a relatively stronger effect on the atria and AVN than on the ventricles.

Generally, the effects of the AF variants on ECG measurements range from none to extensive and vary in direction. For example, AF risk alleles can prolong, shorten, or have no detectable effect on the PR interval, an established predictor of AF development (32). Similarly, there is no clear relationship between the AF effect size and the effect on conduction. For example, the variant in *SCN10A* has extensive and strong ECG effects but small AF effect compared to the strongest common AF variant in *PITX2*. Furthermore, the AF variant with moderate effect in the potassium channel gene *KCNN3* has no association with ECG measurements in the absence of AF. The same applies to several other variants, for example, the novel intergenic AF variants at *LINC01142* /*METTL11B*.

TESTING FOR ASSOCIATION WITH SECONDARY PHENOTYPES. To further explore the p.Gly4098Ser *PLEC* variant, we tested it for association with all phenotypes in deCODE genetics phenotype/genotype database. Apart from AF, the most significant disease associations were with AF-related traits, SSS (OR: 1.64; 95% CI: 1.31 to 2.05; $p = 1.7 \times 10^{-5}$), pacemaker implantation (OR: 1.54; 95% CI: 1.24 to 1.92; $p = 9.5 \times 10^{-5}$), ischemic stroke (IS) (OR: 1.22; 95% CI: 1.01 to 1.47; $p = 0.035$) and the IS subphenotype cardioembolic stroke (OR: 1.53; 95% CI: 1.09 to 2.14; $p = 0.013$) (Online Table 8). Sinus node dysfunction is a common indication for pacemaker implantation and is frequently associated with AF through a complex causal relationship (33), and stroke is a well-known consequence of AF (3). To compare all AF variants, we tested them for associations with these AF-related traits, SSS, pacemaker implantation, IS, and cardioembolic stroke and then plotted the AF risk of each variant relative to the effect on the risk of the AF-related trait (**Figure 3**). The coding AF variant in *MYH6* that was originally discovered through its association to high risk of SSS (13) stood out with substantially greater risk of SSS and pacemaker implantation than predicted from its effect on AF risk. For all other variants, their effects on the AF-related traits were consistent with being proportional to their effects on AF.

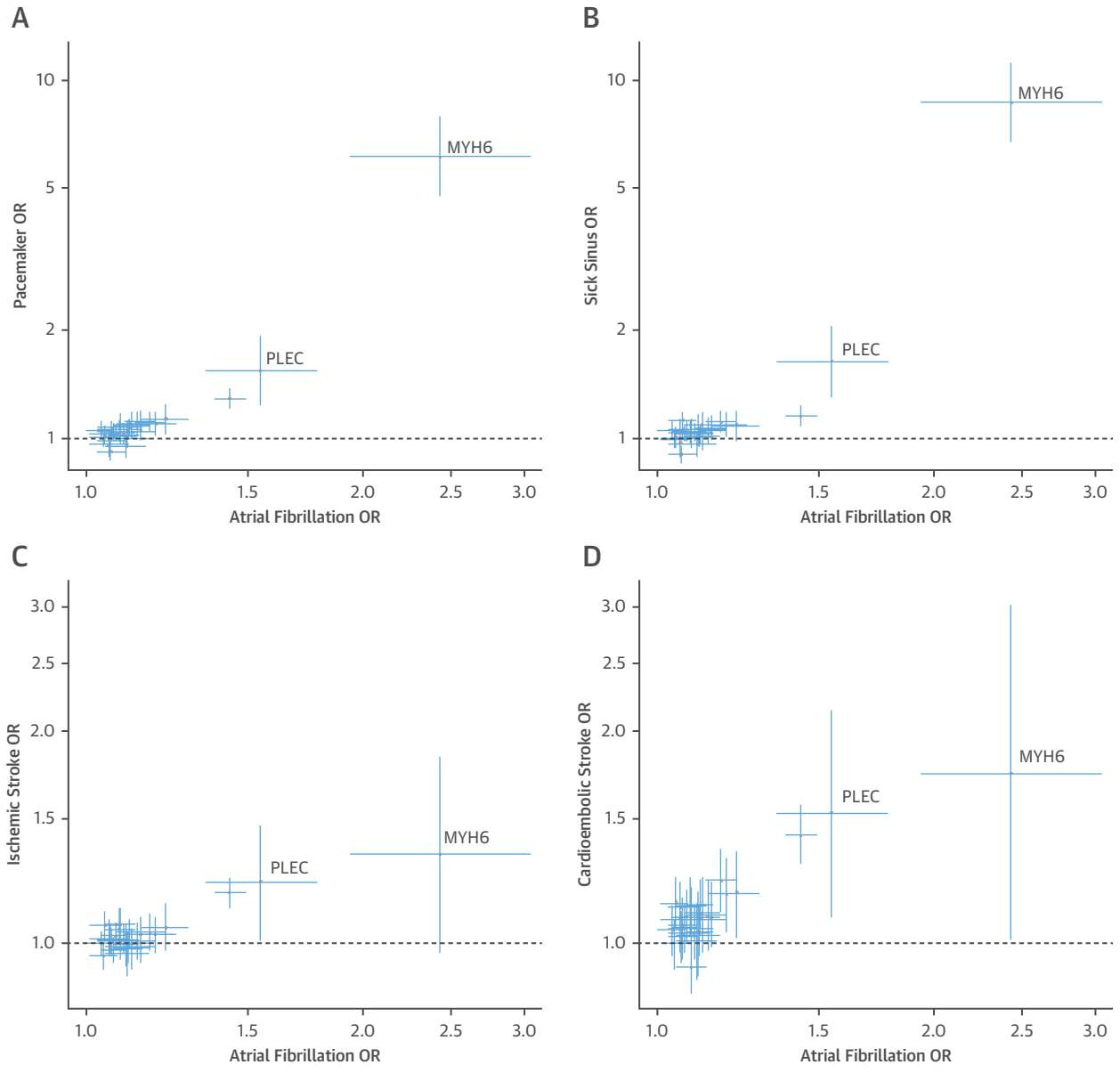
Mutations in *PLEC* have been linked to phenotypes involving skin, muscle, and heart. The association of p.Gly4098Ser in *PLEC*, with available relevant phenotypes including risk factors of AF and some traits described in plectinopathies, are listed in Online Table 8. The only other significant association

FIGURE 2 **Heatmap Shows the Effects of AF Variants on ECG Traits in Sinus Rhythm ECGs, Excluding AF Cases**



Sinus rhythm ECG measurements were available for 62,974 individuals without diagnosis of AF. Each column shows the estimated effect of the risk allele of an AF variant on various ECG traits. The effect of each variant, annotated with the corresponding gene name, is scaled with the $\log_{10}$-AF odds ratio. **Orange** represents a positive effect on the ECG variable, and **blue** represents negative effect. The effect is shown only for significant associations after adjusting for multiple testing with a false discovery rate procedure for each variant. Nonsignificant associations are **white** in the heatmap. AF = atrial fibrillation; ECG = electrocardiogram.

FIGURE 3 AF Effects of AF-Associated Variants on Risk of Pacemaker, SSS, and Stroke



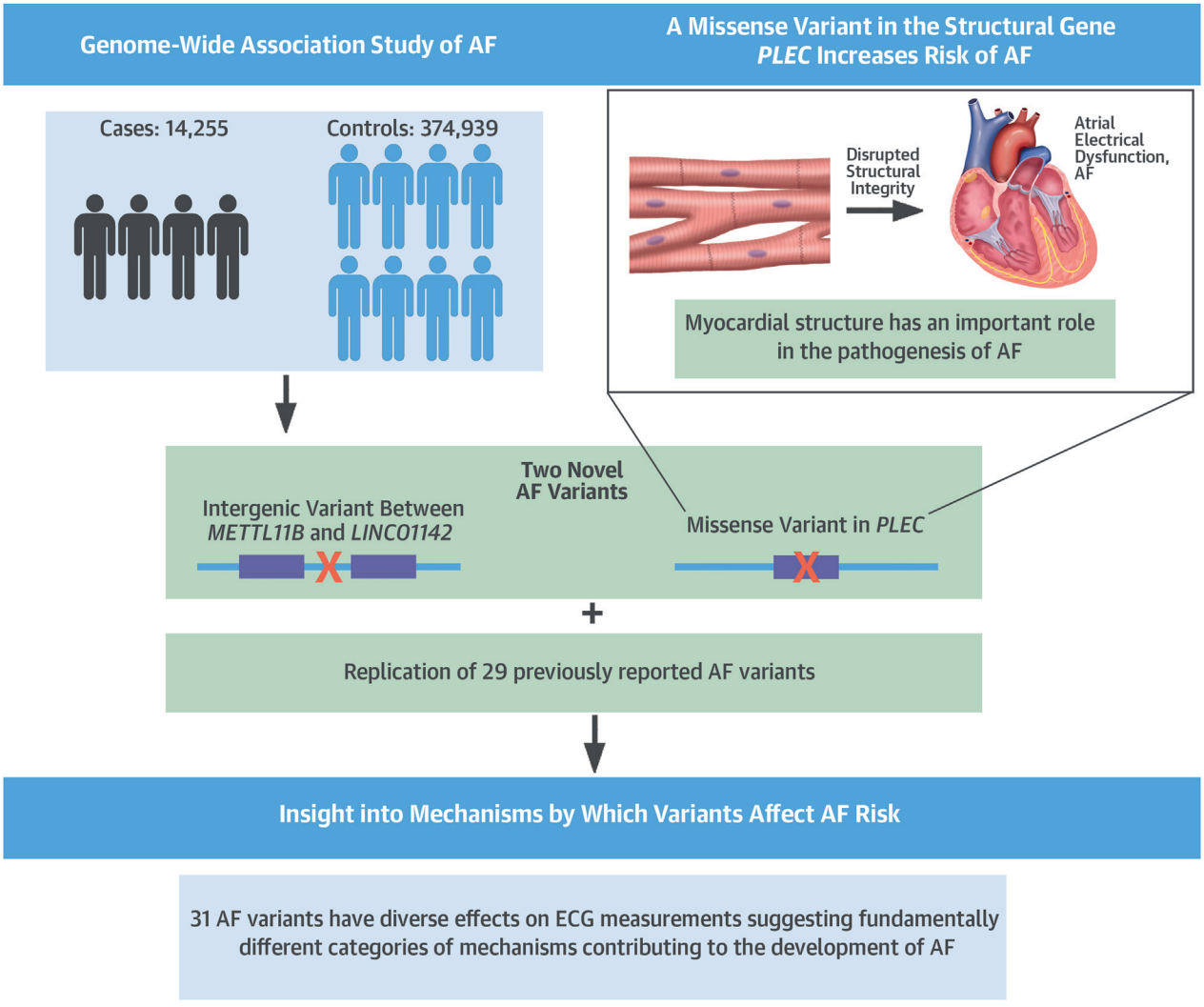
AF effects of AF-associated variants plotted against the effect of **(A)** pacemaker implantation (n = 3,578), **(B)** SSS (n = 3,310), **(C)** ischemic stroke (n = 5,626), and **(D)** cardioembolic stroke (n = 1,369), respectively. A logarithmic scale is used for the axis. AF = atrial fibrillation; OR = odds ratio; SSS = sick sinus syndrome.

($p < 0.05/16 = 0.003$) among these was with lower levels of creatine kinase (beta = −0.09; $p = 2.58 \times 10^{-4}$). Traits described in plectinopathies were not observed from a review of available data for the 17 homozygous carriers of p.Gly4098Ser. Apart from associating with AF and AF-related traits, the other novel AF variant at *LINC01142/METTL11B* was associated with risk of heart failure (OR: 1.10; 95% CI: 1.04 to 1.17; p = 0.0016) (Online Table 9).

DISCUSSION

We performed a large association study based on whole-genome sequencing and identified 2 novel AF variants, a common intergenic variant on chromosome 1q24 and a low-frequency missense variant in the gene *PLEC* (**Central Illustration**). The *PLEC* variant is associated with a 55% increased risk of AF and a 64% increased risk of SSS. It is the third coding

CENTRAL ILLUSTRATION **GWAS of AF and Insight Into Mechanisms by Which Variants Affect Risk for AF**



Thorolfsdottir, R.B. et al. J Am Coll Cardiol. 2017;70(17):2157–68.

This study used whole-genome sequence data to conduct a GWAS of AF (N = 13,471 sequences). We identified 2 novel AF variants and replicated 29 previously reported. Our discovery of a missense variant in *PLEC*, together with our previous findings of rare coding AF variants in 2 myosin genes, *MYH6* and *MYL4*, underscores the role of structural components of the myocardium in the pathogenesis of AF. Furthermore, the study demonstrates the fact that some AF variants have no effect on normal cardiac electrophysiological function, whereas others do. This suggests fundamentally different mechanisms in the development of AF. AF = atrial fibrillation; ECG = electrocardiogram; GWAS = genome-wide association study.

variant found to cause atrial fibrillation in a GWAS and, like the other 2 variants, is in a myocardial structure gene (13,14). This finding underscores the importance of the structural properties of the atria in the pathogenesis of AF.

We also replicated 29 previously reported AF variants and show that in general the variants affect SSS and IS in proportion to their AF effect. On the other hand, the AF variants have highly diverse effects on ECG traits in unaffected individuals, ranging from none or mild to extensive, demonstrating that sequence variants can affect the risk of arrhythmia without substantial disruption of normal cardiac electrical function.

There are several possible mechanisms by which a missense variant in the intermediate filament binding

site of plectin may have structural effects on the heart and cause electrophysiological abnormalities. Studies of plectin$^{-/-}$ mice revealed disintegration of intercalated discs and sarcomere disarrangement, thought to result from lack of plectin in Z-line and intercalated disc structures, to which it connects through intermediate filaments (31,34). Furthermore, the role of plectin in maintaining the integrity of the heart has been demonstrated by observations of cardiac involvement among individuals with homozygous or compound heterozygous *PLEC* mutations (35-39). Homozygous protein-truncating mutations in *PLEC* are known to cause a syndrome of skin fragility, epidermolysis bullosa simplex (EBS), commonly accompanied by muscular dystrophy (EBS-MD) (40,41), myasthenic syndrome (EBS-MDMyS) (42,43), limb-girdle muscular dystrophy type 2Q (LGMD2Q) (44), or pyloric atresia (EBS-PA) (45). Mutations in *PLEC* also cause EBS-Ogna, an autosomal dominant disease without muscular dystrophy (46). Cardiomyopathy and arrhythmias have been described in 5 patients with EBS due to *PLEC* mutations (35-39). An EBS phenotype was not observed in our data for homozygous carriers of p.Gly4098Ser, possibly reflecting the fact that the mutation is not protein-truncating.

p.Gly4098Ser in *PLEC* does not associate with cardiomyopathy in our data, nevertheless association with subclinical or mild cardiomyopathy cannot be excluded. Also, we cannot exclude the possibility that p.Gly4098Ser could cause subclinical or even overt atrial myopathy as this phenotype is not routinely assessed or diagnosed. Indeed, the mutation results in a widespread effect on cardiac electrical function in both atria and ventricles. Furthermore, the association with lower R amplitude and lower creatine kinase levels may reflect less skeletal and cardiac muscle mass of carriers.

Both the previously discovered AF genes, *MYH6* (13) and *MYL4* (14), encode parts of myosin, a major component of the sarcomere, the building block of the muscle contractile system (47). Discovery of their association with AF turned the attention to primary atrial cardiomyopathy as an important cause of this arrhythmia and other clinically significant electrophysiological abnormalities. The discovery of a missense variant in *PLEC* causing AF in the absence of clinical heart failure further supports the theory of clinical electrophysiological consequences of otherwise subclinical mechanical dysfunction. These findings parallel the increasing recognition of atrial cardiomyopathy as a salient entity (48) and an increased awareness of the role of atrial remodeling, defined as any change in atrial structure in the pathogenesis of AF (49). This is exemplified by *MYL4*, where individuals homozygous for a rare frameshift deletion develop AF as early as adolescence (14).

The association between AF variants and ECG traits varies substantially. The 2 coding variants, both in myocardial structural genes, *PLEC* and *MYH6*, have extensive effects on cardiac electrical function, but the *MYH6* variant has a comparatively stronger effect on the atria and AVN than on the ventricles. This is consistent with expression patterns of plectin and αMHC, the protein product of *MYH6*. αMHC expression is restricted to atrial tissue after birth, whereas plectin is equally expressed in atria and ventricles (25,50).

Of the 31 variants we assessed, 29 are in noncoding regions. For most, the genes and molecular mechanisms linking these noncoding variants to risk of AF have not been established. However, many of them are located in or close to genes with a known role in cardiovascular function or development, giving rise to theories on how they affect AF risk. For example, the intronic variant in *SCN10A*, encoding the alpha subunit of a voltage-gated sodium channel, is likely to act through a neighboring sodium channel gene, *SCN5A*. It is in LD with a common variant within *SCN10A* proven to affect expression of *SCN5A*, which is expressed in the adult human heart at 1,000-fold levels higher than its neighboring *SCN10A* (51). This variant widely affects cardiac electrical function. The similarly widespread effect on conduction by the variants in the 2 myocardial structural genes *PLEC* and *MYH6* strongly suggests an important role of cardiac mechanics in maintaining normal electrical conduction. Extensive effect on electrical function is also seen for variants in or close to the developmental genes *NKX2-5* and *TBX5*.

The strongest common AF variant is close to *PITX2* that has a role in cardiac development, and its dysfunction has been linked to atrial structural remodeling (52). Here, the variant only affects indices of atrial and AVN conduction with no observable effect on ventricular conduction. This is consistent with the fact that *PITX2* expression in the ventricles is downregulated during embryogenesis, whereas atrial expression remains high (53), and that atrial but not ventricular chamber-specific deletion of *PITX2* in mice results in atrial electric and structural remodeling linked to arrhythmogenesis (52).

Several AF variants have only mild or no effects on electrocardiogram traits, and there is no clear link between AF effect size and the effect on conduction, highlighting the complex relationship between normal cardiac electrophysiology and arrhythmia development. Clearly, variants can affect the risk of

arrhythmia without substantial disruption of the normal cardiac electrical function. For example, the novel intergenic variant close to *LINC01142* and *METTL11B* does not have an observable effect on conduction. It is unclear by which mechanism this locus affects the risk of AF; there are no obvious candidate genes in the region, and the associated SNPs do not associate with expression of either of the 2 flanking genes in our expression dataset or in that of the Genotype-Tissue Expression project (25).

STUDY LIMITATIONS. The p.Gly4098Ser mutation in *PLEC* is very rare in non-Icelanders and thus we have not been able to demonstrate an AF association for this mutation in populations outside of Iceland. However, this fact does not prevent the generalizability of the discovery to a broader population for several reasons. The *PLEC* gene is already strongly implicated in cardiac function and is the third gene encoding a cardiac structural protein to be linked with AF risk in a genome-wide study (13,14). Furthermore, the mutation associates genome-wide significantly with several ECG variables in the absence of AF, confirming its effects on cardiac conduction. Last, despite this particular variant being largely specific to the Icelandic population, other variants in the *PLEC* gene might be found to affect AF in other populations as was the case for *MYL4* (54).

CONCLUSIONS

We describe the discovery of the third rare or low-frequency coding variant conferring risk of AF in a GWAS. Of interest, all 3 variants were found in genes that affect myocardial structure. This underscores the role of structural components of the myocardium in the pathogenesis of AF and supports the notion that otherwise subclinical mechanical dysfunction may have clinical electrophysiological consequences. Coding variants in the structural genes *PLEC* and *MYH6* associate with AF, and both widely affect ECG measurements in the absence of AF. Other GWAS AF variants that we assess are in noncoding regions, and the mechanism by which they affect AF risk is largely unknown. Here we demonstrate a marked diversity in their effects on normal cardiac electrical function and, in some cases, a lack of such an effect, underscoring the extreme complexities in the pathogenesis of this common arrhythmia.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Coding variants in myocardial structural genes increase the risk of developing atrial fibrillation, suggesting a role for cardiac mechanics in maintaining electrical homeostasis. Maintaining normal cardiac structure is important for prevention of AF, and sequence variants can raise the risk of arrhythmia with little or no substantial disruption of normal cardiac electrical function.

TRANSLATIONAL OUTLOOK: Further studies are needed to elucidate the mechanisms by which sequence variants raise the risk of developing AF.

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KEY WORDS arrhythmia, atrial fibrillation, electrocardiogram, plectin

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this article.