JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 7, 2024

STATE-OF-THE-ART REVIEW

Genetics and Pharmacogenetics of Atrial Fibrillation

A Mechanistic Perspective

Asia Owais, MBBS, MS,[a] Miles Barney, BA,[a] Olivia Thao Ly, MS,[a,b] Grace Brown, PhD,[a,b] Hanna Chen, MD, PhD,[a] Arvind Sridhar, MS,[a] Arif Pavel, PhD,[a] Salman R. Khetani, PhD,[b] Dawood Darbar, MD[a,b,c,d]

HIGHLIGHTS

- **The diversity of genes and biological pathways involved in AF pathogenesis and variability in individual response to treatment highlights the need for a personalized approach for AF management. Over the past decade, GWAS have identified more than 140 AF-associated loci, and family-based studies have identified rare variants in genes encoding ion channel as well as non-ion channel proteins.**
- **This review discusses studies that have provided novel insights regarding variant-specific pathophysiologic mechanisms that can pave the way for personalized medicine for AF.**
- **Recent translational advances in AF genetics include the development of high-throughput in vitro assays for functional validation of variants of uncertain significance that can bridge the gap in variant classification and risk stratification.**
- **GWAS-derived PRS for AF can be used for predicting AF risk and adverse outcomes. Further studies are needed to validate if AF PRS are superior to traditional risk stratification tools.**
- **Genotype-guided clinical trials and advances in bioengineered preclinical models are needed for developing personalized therapy for AF.**

SUMMARY

The heritability of atrial fibrillation (AF) is well established. Over the last decade genetic architecture of AF has been unraveled by genome-wide association studies and family-based studies. However, the translation of these genetic discoveries has lagged owing to an incomplete understanding of the pathogenic mechanisms underlying the genetic variants, challenges in classifying variants of uncertain significance (VUS), and limitations of existing disease models. We review the mechanistic insight provided by basic science studies regarding AF mechanisms, recent developments in high-throughput classification of VUS, and advances in bioengineered cardiac models for developing personalized therapy for AF.
(JACC Basic Transl Sci 2024;9:918–934) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

From the [a]Division of Cardiology, Department of Medicine, University of Illinois, Chicago, Illinois, USA; [b]Department of Biomedical Engineering, University of Illinois, Chicago, Illinois, USA; [c]Department of Physiology and Biophysics, University of Illinois, Chicago, Illinois, USA; and the [d]Department of Pharmacology and Regenerative Medicine, University of Illinois, Chicago, Illinois, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received August 31, 2023; revised manuscript received December 11, 2023, accepted December 12, 2023.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.12.006

Atrial fibrillation (AF), the most common cardiac arrhythmia requiring therapy, is associated with significant morbidity and increased mortality.[1] Epidemiologists predict that in the next 3 decades, the incidence of AF may rise to 16 million in the United States alone.[2] Despite recent advances in catheter-based ablation therapy, AF recurs in as many as 40% of patients after ablation, and antiarrhythmic drug (AAD) therapy fares even worse, with ~50% of patients experiencing a recurrence of AF within 6 months.[3,4] The variability in response to treatment is not only determined by the underlying genetic heterogeneity but also by the type of AF (paroxysmal vs persistent vs long-standing persistent), with more advanced forms showing greater resistance to maintenance of sinus rhythm.[4,5] Despite the advances in AF genetics, personalized therapy for AF has not yet been translated into clinical practice. A fundamental obstacle to improving AF treatment through personalized medicine is incomplete understanding of the underlying genetic and molecular substrate of AF and our inability to predict response to therapy.[5]

Over the past 2 decades, tremendous progress has been made in understanding the genetic architecture of AF. Genome-wide association studies (GWAS) have identified more than 140 AF-associated loci.[5] In contrast, linkage analysis and whole exome sequencing have implicated rare mutations encoding ion channels, signaling molecules, transcription factors, and, recently, structural proteins linked with familial or early-onset AF. The mechanisms underlying these genetic variants are under investigation to develop personalized therapies tailored to the cellular and molecular substrate.

The large amount of genomic data acquired from GWAS is being used to develop polygenic risk scores (PRS) to determine AF risk.[6] Whether AF PRS can guide clinical care decisions to identify subclinical AF and prevent and reduce AF-associated morbidities remains unclear. One of the translational challenges in AF genetics is the large proportion of variants of unknown clinical significance (VUS) identified by genetic studies. High-throughput in vitro assays are being developed for reclassification and screening of VUS.[7,8] The broad array of genetic risk factors associated with AF not only shows the complex and heterogeneous substrate but also highlights the limitations of in vitro heterologous expression systems and in vivo animal models to adequately recapitulate AF pathophysiology that translate to the bedside care of patients. Primary cell culture has the distinct advantage of being patient specific, but human atrial tissue is rarely accessible and these cell lineages have limited survival. Human-induced pluripotent stem cells (hiPSCs) are an extremely versatile and powerful tool that address many of the limitations of existing in vitro and in vivo models and have several advantages for translational studies in AF. For example, despite the cardiac ion channels being highly conserved among mammals, the individual currents shaping the cardiac action potential (AP) vary considerably across species.[9] Using cardiomyocytes from hiPSCs (hiPSC-CMs) therefore provides higher fidelity for electrophysiologic (EP) assays. Furthermore, hiPSCs are easily derived from fibroblasts or peripheral blood mononuclear cells, making patient-specific hiPSC lines for mechanistic studies and drug screening feasible.[10] Finally, genome editing in hiPSCs has allowed for a greater understanding of the genetic mechanisms of AF at the molecular and cellular levels.

In this paper we review the current understanding of the pathophysiologic mechanisms of both common and rare AF-associated variants, with a focus on recent studies that have identified new AF loci and putative candidate genes; the variability in response to therapy; the current status of AF PRS in risk stratification; translational advances in functional genomic screens for VUS and hiPSC-based cardiac models; and the current and future perspective on clinical applications of genetics in AF management.

ABBREVIATIONS AND ACRONYMS

AAD = antiarrhythmic drug
AF = atrial fibrillation
AP = action potential
APD = action potential duration
DAD = delayed afterdepolarization
EAD = early afterdepolarization
EHT = engineered heart tissue
EP = electrophysiologic
GOF = gain-of-function
GWAS = genome-wide association studies
hiPSC-aCM = human-induced pluripotent stem cell-derived atrial cardiomyocyte
LOF = loss-of-function
PRS = polygenic risk score
SNP = single nucleotide polymorphism
VUS = variants of uncertain clinical significance

GENETIC MECHANISMS OF AF

There is a significant heritability to AF, estimated to be up to 22% in people of European ancestry.[4,5] Common variants identified by GWAS have a minor allele frequency of ≥1%, and rare variants, which are far less common, have a minor allele frequency of ≤1%. Although common variants provide an estimate of disease susceptibility, they are not necessarily causative. In contrast, rare pathogenic variants can be causative and have higher penetrance and much lower frequency. A recent study performed on 1,293 patients from the Vanderbilt AF registry, identified patients with rare pathogenic variants in arrhythmia and cardiomyopathy genes in 10.1% of participants with early-onset AF (AF diagnosis before 66 years of age).[11] Among the most prevalent pathogenic variants, 27% were in *TTN*, 13% in *MYH7*, 6% in *LMNA*, 7% in *MYH6,* and 6% in *KCNQ1*.[11]

The diversity of genes and biological pathways contributing to AF risk suggests that the underlying

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 7, 2024
JULY 2024:918–934

pathophysiology is highly heterogeneous. Such heterogeneity emphasizes the importance of a personalized approach to diagnosis, assessment, treatment, and genetic counseling.[5] We now discuss the mechanisms underlying common and rare AF-associated variants in ion and nonion channel proteins.

ION CHANNEL VARIANTS. Sodium channel variants. Voltage-gated sodium channels initiate and maintain AP activation and impulse propagation. The most abundant sodium channel, encoded by the *SCN5A* gene, is the α-subunit of Nav1.5.[12] Atrial depolarization on the electrocardiogram, represented by the P-wave, is determined by both sodium and calcium channels.[13] Mutations in *SCN5A* may be either gain-of-function (GOF) or loss-of-function (LOF), exhibiting increased sustained sodium current (I_{Na}) or compromised peak I_{Na}.[12] GOF mutations induce AF by increasing AP duration (APD) and cellular excitability (**Central Illustration**, **Table 1**); long QT syndrome is another example of a channelopathy caused by an *SCN5A* GOF mutation.[14] LOF mutations induce AF by reducing conduction velocity.

The second major cardiac sodium channel is Nav1.8, encoded by *SCN10A*, which until recently, was thought to be expressed only in the sensory neurons of the dorsal root ganglion in the peripheral nervous system. The voltage-gated sodium channel Nav1.8 is responsible for the late sodium current, $I_{Na,L}$. Blocking Nav1.8 in cardiomyocytes reduces $I_{Na,L}$ and shortens the APD. Both GOF and LOF *SCN10A* mutations are associated with early-onset AF.[15] *SCN10A* may modulate *SCN5A* physiologically and pathologically,[16] and it is currently unknown if SCN10A functions independently from SCN5A.

Characterization of a pathogenic GOF variant *SCN5A*-E428K with the use of hiPSC-derived atrial cardiomyocytes (aCMs) revealed increased $I_{Na,L}$, which was inhibited by ranolazine.[17] Transcriptomic studies showed an up-regulation of the nitric oxide pathway with enhanced nitrosylation of Nav1.5, which modulated $I_{Na,L}$ and triggered AF. Although the signaling pathways regulating $I_{Na,L}$ are complex, it is possible that the *SCN5A*-E428K GOF mutation leads to increased calcium-calmodulin kinase II phosphorylation of the cardiac sodium channel and enhances $I_{Na,L}$.[18] A recent study using iPSC-CMs derived from carriers of a common variant, *SCN5A* S1103Y, which is found at a higher frequency in individuals with African and East Asian ancestry, showed enhanced $I_{Na,L}$ with a normal APD. The normal APD was attributed to an increase in the rapid component of delayed rectifier potassium current, I_{Kr}. An I_{Kr} blocker, dofetilide, prolonged the APD at a much lower dose in S1103Y iPSC-CMs, revealing their susceptibility to torsadogenic drugs.[19]

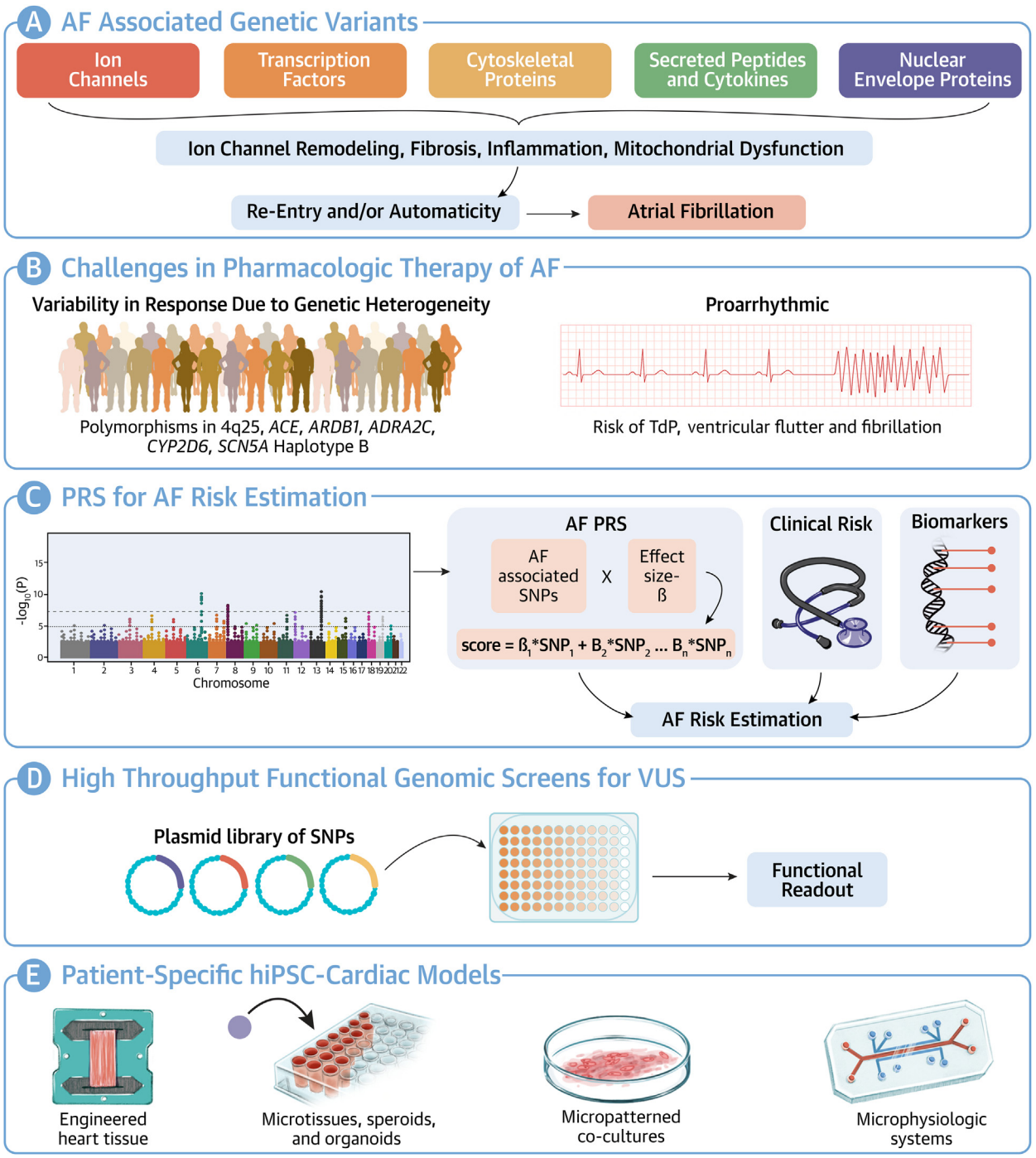
Potassium channel variants. Cardiomyocytes express several types of potassium channels, which are responsible for achieving and maintaining a sufficiently stabilized and hyperpolarized membrane, with each playing a different role in repolarization. The ultrarapidly repolarizing potassium (I_{Kur}) channel Kv1.5, encoded by *KCNA5*, is expressed almost exclusively in aCMs. As an atrial-specific channel, it is a promising therapeutic target for AF.[20] Nonsense *KCNA5* mutations increase susceptibility to AF, as well as electrical and conduction instability. Loss-of-function mutations result in delayed AP repolarization, prolonged APD, and early afterdepolarizations (EADs) (**Figure 1**, **Table 1**).[20] GOF mutations shorten APD and increase atrial excitability, triggering AF.[21]

The first gene (*KCNQ1*) linked with familial AF, encoding the cardiac delayed rectifier potassium channel current (I_{Ks}), was identified in a large Chinese kindred with early-onset AF.[22] Both GOF and LOF mutations in *KCNQ1* have been associated with early-onset AF through the modulation of the APD and refractory period and enhanced atrial AP repolarization.[22] GOF mutations in *KCNE2,* one of the subunits of *KCNQ1,* are also associated with AF via increased I_{Ks} and enhanced AP repolarization.[24]

A GOF mutation in the gene (*KCND3*) encoding the transient outward potassium channel Kv4.3 and current (I_{to}) also has been associated with early-onset AF.[25] *KCNJ2* encodes the inward rectifier potassium channel, Kir2. There are 5 different members of Kir2. Kir2.1 is found mainly in the ventricles and regulates the resting membrane potential. In contrast, Kir2.3 is primarily expressed in aCMs.[26] A gain-of-function in *KCNJ2* results in increased I_{K1} as well as enhanced atrial AP repolarization.[24,27] In 2010, a GWAS conducted by Ellinor et al[28] identified a single nucleotide polymorphism (SNP) in the gene *KCNN3*, a gene encoding calcium-activated potassium channel SK3. Calcium-activated potassium channels of small conductance (SK1-3) are an emerging atrial-specific target discussed below.

Calcium channel variants. The L-type calcium channel Cav1.2, encoded by *CACNA1C*, is the dominant ion channel in excitation-contraction coupling in the heart, playing a crucial role in phase 2 of the AP and contributing to the EP profile of cardiomyocytes. Whereas Cav1.2 is the only voltage-gated calcium channel in ventricular cardiomyocytes, Cav1.3 (encoded by *CACNA1D*), is found in atrial myocytes, nodal cells, and vascular smooth muscle cells.[14] Cav1.3 knockout mice show impaired calcium homeostasis, which predisposes to AF.[29] Furthermore,

CENTRAL ILLUSTRATION **Genetic Mechanisms of AF and Translational Outlook**



Owais A, et al. JACC Basic Transl Sci. 2024;9(7):918–934.

(A) Atrial fibrillation (AF)-associated variants identified in genes encoding ion channels, transcription factors, cytoskeletal proteins, secreted peptides, and cytokines, and nuclear envelope proteins create an arrhythmogenic substrate for AF by various mechanisms. (B) Challenges in pharmacologic therapy of AF include genetic heterogeneity leading to variability in drug responses and proarrhythmic effects. (C to E) Translational outlook in AF: (C) polygenic risk scores (PRS) for AF—calculated by the weighted sum of the product of AF-associated single nucleotide polymorphisms (SNPs) and their effect size—are complementary to clinical risk and AF biomarkers in predicting AF risk; (D) high-throughput functional genomic screens for assessing variants of uncertain clinical significance (VUS) in AF-associated genes; (E) bioengineered models of patient-specific human induced pluripotent stem cell (hiPSC)-derived cardiac models for understanding pathophysiologic mechanisms and testing drug responses. TdP = torsades de pointes.

TABLE 1 Genetic Mechanisms of Atrial Fibrillation

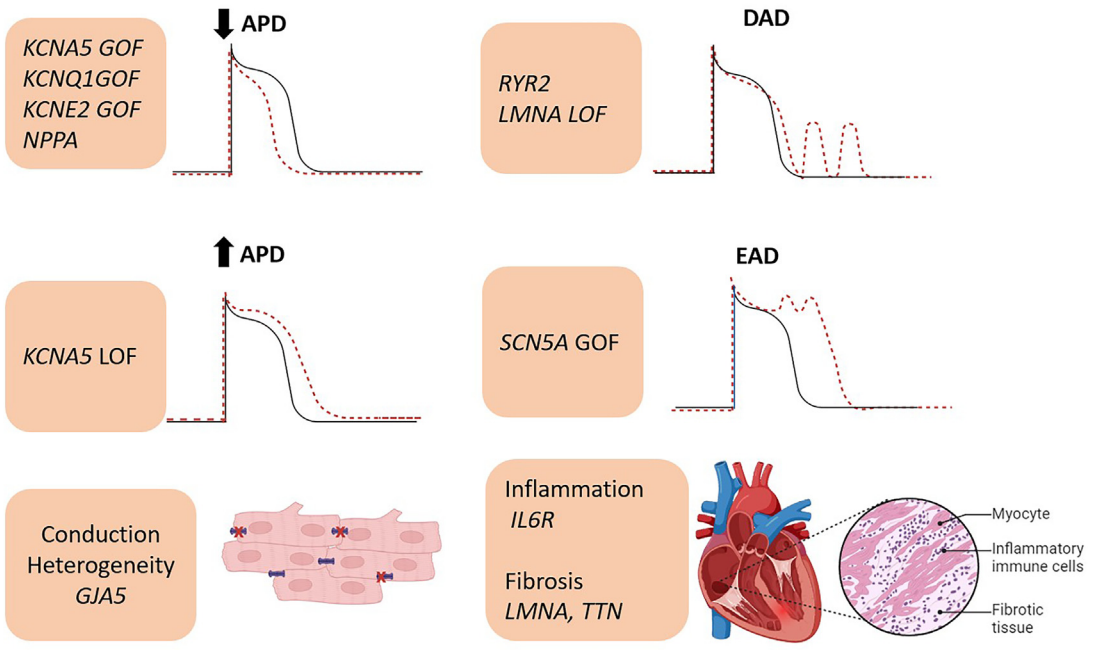
Gene	Protein	Mechanism	First Author
Ion channel variants			
Sodium channel			
SCN5A GOF	Nav1.5	EADs	Remme and Bezzina[12]
SCN5A LOF, *SCN10A* LOF	Nav1.5, Nav1.8	Reduction in conduction velocity	
Potassium channel			
KCNA5 LOF	Kv1.5	APD prolongation	Chen et al,[22] Mahida et al,[23] and Brugada et al[24]
KCNA5 GOF	Kv1.5	Shortening of APD	
KCNQ1 GOF	Kv1.7	Shortening of APD	
Calcium channel			
CACNA1D LOF	-	Impaired calcium homeostasis, regulation of ANP and BNP secretion	Mancarella et al[29] and Srivastava et al[30]
RYR2	Ryanodine receptor 2	Triggered calcium release	Zhabyeyev et al[32]
GJA5	Connexin-40	Conduction heterogeneity leading to reentry	Gollob et al[33]
Non-ion channel variants			
Transcription factors			
PITX2	Paired homeobox transcription factor 2	Increased activity of RyR channels Slight prolongation of APD Regulates transcription of genes encoding ion channels	Zhang et al[38] and Kim et al[43]
ZFHX3	Zinc finger homeobox 3	Structural remodeling, repression of *SCN5A*	Rubio-Alarcón et al[45]
TBX3	T box transcription factor 3	Increased ectopic automaticity	Hoogaars et al[55]
TBX5	T box transcription factor 5	Regulation of transcription of AF-associated genes	Hiroi et al[57]
GATA4, *GATA6*	GATA-binding protein 4	Abnormal development of pulmonary vein myocardium Impaired regulation on downstream AF-associated target genes, such as *NPPA*[1]	Roselli et al[46] and Wang et al[47]
NKX2.5	NK2 homeobox 5	Delayed conduction	Chen et al[49] and Shiratori et al[50]
ERRg	Estrogen receptor γ	Regulates the expression of AF-associated genes	Miyazawa et al[58]
Secreted peptides			
NPPA	ANP	Shortening of APD	Hodgson-Zingman et al[76]
Cytoskeletal proteins			
TTN	Titin	Sarcomeric dysfunction, fibrosis	Ahlberg et al[67]
MYL4	Myosin light chain 4	Part of ATPase cellular motor protein complex	Nattel et al,[61] Gudbjartsson et al,[62] and Goette et al[63]
MYH7	Myosin heavy chain 7	Sarcomeric dysfunction	Maron et al[70]
MYBPC3	Myosin-binding protein C3	Sarcomeric dysfunction	Maron et al[70]
DES	Desmin	Intermediate filament protein, reduced AERP, conduction delay, mitochondrial dysfunction	Schrickel et al[72]
PLEC	Plectin	Cytoskeletal structure disruption	Thorolfsdottir et al[74]
JPH2	Junctophilin	Tethering protein in excitable cells, abnormal calcium release binds to RyR	Beavers et al[75]
Nuclear envelope proteins			
LMNA	Lamin A/C	Triggered DADs	Salvarani et al[82]
NUP155	NUP155	APD shortening, impaired nucleocytoplasmic transport	Zhang et al[83]
Mediators of inflammation			
IL6R	Interleukin-6 Receptor	Cytokine receptor promotes inflammation Predicts AF recurrence after catheter ablation	Miyazawa et al[58]

ANP = A-type natriuretic peptide; APD = action potential duration; ATPase = adenosine triphosphatase; BNP = B-type natriuretic peptide; DAD = delayed afterdepolarization; EAD = early afterdepolarization; GOF = gain-of-function; LOF = loss-of-function; RyR = ryanodine receptor.

Cav1.3 regulates the secretion of A- and B-type natriuretic peptides.[30] L-type calcium channel blockers are frequently used as rate control therapy for AF, with limited toxicity or debilitating side-effects.[31]

Ryanodine receptors are active during calcium-induced calcium release. After membrane depolarization activates L-type calcium channels on the surface of the sarcoplasmic reticulum, an initial calcium influx triggers a second wave of calcium release through the ryanodine receptors, thus resulting in the complete contraction of sarcomeres. Mutations in ryanodine receptors cause automaticity, characterized by calcium-triggered delayed afterdepolarizations (DADs) (**Figure 1**, **Table 1**).[25,32]

FIGURE 1 Mechanisms of Atrial Fibrillation-Associated Genetic Variants



Genetic variants create an arrhythmogenic substrate by different mechanisms, including alteration of action potential duration, cell-to-cell coupling, fibrosis, and inflammation. APD = action potential duration; DAD = delayed afterdepolarization; EAD = early afterdepolarization; GOF = gain-of-function; LOF = loss-of-function.

Gap junction mutations. Connexins are gap junction proteins that play an important role in cell-cell communication, syncytial electrical coupling, and impulse propagation in the human myocardium. Thus, mutations in *GJA5* (encoding the atrial-specific gap junction protein connexin-40) could lead to heterogeneous cardiac conduction, impaired propagation of an electrical impulse between adjacent cardiomyocytes, and increased AF susceptibility (**Figure 1**, **Table 1**).[33] Several studies have reported the association of both common and rare germline variants and somatic mutations in *GJA5* with AF susceptibility and pathogenesis, respectively.[34-36]

NONION CHANNEL VARIANTS. Traditionally, AF is considered to be a channelopathy, but over the past decade, GWAS as well as candidate gene studies have identified an increasing number of variants in non-ion channel genes that are associated with AF. Variants in cytoskeletal proteins, cardiac hormones, nuclear envelope proteins, and transcription factors have been linked to AF pathogenesis. Here, we discuss non-ion channel variants that are associated with AF and the underlying molecular mechanisms that create an arrhythmogenic substrate for AF.

Transcription factors. GWAS have identified a significant genetic signal on chr4q25, an intergenic region with at least 4 independent AF-associated loci near the *PITX2* gene.[37] Although nonhuman animal models suggest that PITX2 plays a key role in AF pathogenesis, it was only recently that chromosome conformation capture studies showed that chr4q25 SNPs interact with the promoter of the cardiac-specific isoform of *PITX2C*, supporting the hypothesis that 4q25 variants regulate *PITX2* expression.[38] In adult human hearts, *PITX2C* is confined to the left atrium and targets genes encoding ion channels (*CACNA1D*, *CACNA2D2*, *RYR2*, *ATP2A2*, *JPH2*, *KCNQ1*, *KCNN3*, *KCNJ11*), transcriptional regulators (*TBX20*, *HDAC7*, *ZFHX3*), and intercalated disk proteins (*GJA1*, *CTNNB1*).[39] Deficiency in *PITX2* increases susceptibility to AF.[40] PITX2C is required for the formation of the pulmonary myocardium, which is a source of triggered electrical activity in AF. In paroxysmal AF, pulmonary vein isolation is the main ablation

strategy.[41] PITX2 is also regulated by gremlin-2, encoded by *GREM2*, which is a bone morphogenetic protein antagonist and is associated with AF. Increased expression of gremlin-2 has led to decreased contraction and slower conduction velocity.[25,41]

A recent study on PITX2$^{-/-}$ atrial engineered heart tissue (aEHT) recapitulated some of the electrical remodeling in AF. PITX2$^{-/-}$ aEHT had a triangulation of APD and a slight increase in APD90. That supported the use of aEHT for studying the effects of specific variants on ion channel electrophysiology in AF.[42] In addition to potassium channel remodeling, haploinsufficient mouse models of PITX2$^{+/-}$ have implicated hyperactivity of ryanodine receptor channels in PITX2-mediated AF pathogenesis.[43] *ZFHX3* (zinc finger homeobox3) is the second most common gene associated with AF.[44] It encodes for a transcription factor required for right-left atrial patterning and predisposes to atrial cardiomyopathy. A recent study showed that ZFHX3 regulates the expression of the sodium channel and peak I_{Na}.[45]

A multiethnic GWAS conducted by Roselli et al[46] identified loci close to genes encoding transcription factors TBX3, TBX5, and NKX2-5 with AF. These genes play an important role in the development of the cardiac conduction system.[47] NKX2 insufficiency caused the up-regulation of HCN4 channels, down-regulation of CX40, and disruption of calcium handling proteins in cultured atrial cells, revealing the molecular substrate for AF.[48,49] Furthermore, NKX2.5 regulates the expression of *PITX2* by binding to the enhancer element of PITX2. The 2 transcription factors may work in concert to regulate the expression of genes involved in AF pathogenesis.[50]

Variants in *GATA-4* and *GATA-6* transcription factors genes have been found to co-segregate with AF.[47,51] This may be partially explained by impaired development of pulmonary sleeve myocardium as well as impaired regulation of transcription of AF-associated genes such as *ANP*.[50] Rare variant joint analysis as well as GWAS studies have associated *PRRX1* with AF.[52,53] PRRX1 regulates transcription factors involved in smooth muscle development.[54] Abnormal smooth muscle morphogenesis of pulmonary vasculature leading to ectopic electrical activity may be a potential link between *PRRX1* variants and AF.[48,54]

T-box transcription factors *TBX3* and *TBX5* have been associated with AF.[46] TBX3 regulates the sinoatrial node gene program and may be involved in abnormal ectopic automaticity in AF.[55] TBX5 is involved in cardiac development and limb identity. Variants in TBX5 are associated with AF and cause Holt Oram syndrome, which is characterized by congenital heart defects, conduction disease, and upper limb deformities. The synergistic action of TBX5 and NKX2 regulates the transcription of AF-related genes such as *NPPA*, *KCNJ2*, *CX40*, and *TBX3*.[56,57]

More recently, a cross-ancestry GWAS of more than 1 million individuals conducted by Miyazawa et al[58] analyzed transcription factors that bound to AF loci and found substantial enrichment of estrogen receptor γ at AF loci. Functional studies in hiPSC-CMs revealed that pharmacologic inhibition of estrogen receptor γ caused irregular beating, prolongation of contraction duration, and decreased expression of AF-associated genes. Transcriptome-wide analysis implicated *IL6R* to be a causal gene that is supportive of the role of inflammation in AF pathogenesis.[58]

Cytoskeletal proteins. We and others have recently identified rare mutations in myocardial sarcomeric proteins associated with early-onset AF.[37,59,60] Increasingly, atrial myopathy, ie, any structural, macro- or cyto-architectural, contractile, fibrotic, or EP remodeling in the atria is being recognized as a potential cause of AF.[61-63] Atrial dilation may be secondary to increased ventricular filling pressures, creating a substrate for AF. However, hemodynamic consequences of ventricular cardiomyopathy may not always be responsible for AF. Increasingly, mutations in genes encoding sarcomeric proteins have been associated with early-onset AF. A mutation in the gene that encodes for the atrial-specific sarcomeric protein myosin light chain 4 (*MYL4*) was the first gene linked with AF due to an atrial myopathy.[60] MYL4 is part of an adenosine triphosphatase cellular motor protein complex. Loss of MYL4, resulting in highly penetrant AF, suggests the involvement of the contractile apparatus in impulse conduction, with or without an associated cardiomyopathy.[62] Increasingly, genes implicated in ventricular cardiomyopathy are being recognized as a cause of AF. Zhang et al[64] reported a family with AF harboring a pathogenic mutation in *LMNA*. A recent study compared echocardiographic parameters of patients with *LMNA* and truncating variants in *TTN* (*TTNtvs*) with healthy control subjects and found evidence of atrial myopathy in patients harboring *LMNA* variants reflected by reduced left atrial contractile strain in the absence of left ventricular dysfunction and left atrial dilation.[65]

The sarcomeres serve as the bridge between mechanical and electrical functions in the heart. The Z-disk of sarcomeres links excitation with contraction, communicating electrical impulses to the sarcomere by means of the deep invaginations of the T-tubule

system, which is densely packed with L-type calcium channels.[66] Titin, which connects the thick filament of the sarcomere to the Z-disks, is expressed in all chambers of the heart. Heterozygous *TTNtvs* are responsible for about 20% of all dilated cardiomyopathy and have also been associated with an increased burden of AF.[59,67] *TTNtvs* result in compromised cardiac performance and metabolism, and one-third of patients with these variants develop heart failure within 5 years of AF diagnosis.[68] AF development following heart failure onset is also common.[69] This co-occurrence of heart failure and AF suggests that sarcomeric dysfunction could be a common pathophysiologic link with early-onset AF.[58] A zebrafish model of a rare variant in *TTN* showed atrial fibrosis in larval and adult zebrafish, highlighting the potential role of fibrosis in the pathogenesis of AF.[67] Variants associated with hypertrophic cardiomyopathy, such as *MYH7* and *MYBPC3*, are also associated with AF, but it remains unclear why there is a higher incidence of AF associated with these variants.[70]

Desmin is an intermediate filament protein encoded by the *DES* gene. Variants in desmin increase the risk for cardiac arrhythmias.[71] Studies in mouse models and hiPSC-CMs have provided insights into the molecular mechanism underlying desmin-associated cardiomyopathy and arrhythmogenesis. Desmin knockout mice have prolonged interatrial conduction and an increase in supraventricular as well as ventricular ectopic beats and a reduction in the atrial effective refractory period.[72] Desmin aggregates hamper mitochondrial function by altering mitochondrial respiration. Interestingly, mitochondrial dysfunction in mouse models has been observed before the onset of cardiac pathology.[73] *PLEC* encodes a large cytoskeletal protein. A GWAS on an Icelandic cohort identified variants in *PLEC* to be associated with AF.[74]

Variants in *JPH2*, encoding junctophilin, a structural protein linking L-type calcium channels in T-tubules with ryanodine receptors, were found in 2 patients with juvenile-onset AF. A knockin mouse model of this variant had increased inducibility of AF and abnormal calcium release events. This was attributed to decreased binding of JPH2 with ryanodine receptors revealing the molecular mechanism underlying *JPH2*.[75]

Cardiac hormone: A-type natriuretic peptide. Next-generation sequencing has identified common and rare genetic variations in genes that encode A-type natriuretic peptide (ANP), NPPA, and the renin-angiotensin-aldosterone system. ANP is crucial in controlling intravascular blood volumes, vascular tone, vasodilation, and diuresis, as well as modulating sodium, potassium, and calcium channels. The protein that results from a frame-shift mutation in *NPPA* shortened the atrial monophasic AP, potentially predisposing to a reentrant mechanism of AF.[76] GOF mutations in both *KCNQ1* and *NPPA* shortened APD and altered calcium handling.[77] Furthermore, mutations in angiotensin, angiotensinogen, and angiotensin-converting enzymes cause atrial fibrosis and structural remodeling, resulting in electrical heterogeneity and a reentrant mechanism of AF.[76,77] Ly et al[77] modeled an *NPPA* variant in hiPSC-aCMs that showed a shortening of APD and an increase in the delayed rectifier potassium current (I_{Ks}).

Nuclear envelope proteins. *LMNA* encodes the nuclear envelope proteins lamin A/C, which play an important role in maintaining the nuclear and cytoskeletal architecture, gene expression, and transcriptional regulation.[78] Pathogenic variants in *LMNA* cause malignant heart disease characterized by conduction disease, atrial and ventricular arrhythmias, and dilated cardiomyopathy.[78,79] AF is the most common arrhythmia in lamin A/C heart disease and is often the first clinical presentation in the absence of overt cardiomyopathy.[80] Studies from rodent models have implicated mitogen-activated protein kinase (MAPK), mammalian target of rapamycin, and increased activity of transforming growth factor-β pathways underlying the cardiomyopathy in lamin A/C heart disease, with hiPSC-CMs providing important insights into arrhythmogenic mechanisms. hiPSC-CMs derived from a frameshift mutation in *LMNA*-K117fs showed increased activity of the transforming growth factor β pathway underlying the phosphorylation of sarcoplasmic reticulum calcium channel and ryanodine receptor, leading to spontaneous calcium release and DADs.[81] Lamin A/C interacts with chromatin in defined regions known as lamin-associated domains. Mutations in lamin A/C can cause changes in lamin-associated domains as well as spatial organization of chromatin, leading to altered expression of genes. hiPSC-CMs derived from a family affected by a missense variant K219T in *LMNA* revealed epigenetic silencing of the *SCN5A* gene that led to a decrease in peak I_{Na} as well as diminished conduction velocity.[82] Variants in *NUP155,* which encode nucleoporins, co-segregate with AF and sudden death in early childhood.[83,84] Studies in mouse models have shown the shortening of APD and impaired nucleocytoplasmic transport as the underlying mechanism in AF.

An incomplete understanding of the genetic mechanisms by which genetic variants in *TTN*, *MYL4*, and other nonion channel genes cause AF limits

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 7, 2024
JULY 2024:918–934

pharmacologic therapy for patients harboring these mutations as current AADs solely target ion channels. Elucidating the underlying molecular mechanisms by which mutations in structural genes result in AF will not only identify novel therapeutic targets, but also enable a mechanism-based approach to the treatment of this common and morbid arrhythmia. The **Central Illustration** lists the genes associated with AF. Mechanisms of AF underlying genetic variants are illustrated in **Figure 1**.

GENOTYPIC DIFFERENCES IN RESPONSE TO THERAPY

RESPONSE TO AADs. The first pharmacogenetic study that supported the idea of genotype-guided response to AADs assessed the *ACE I/D* polymorphism.[85] *ACE* I and D correspond to the insertion or deletion, respectively, of a 287-base-pair intronic segment in the *ACE* gene. Patients with *ACE* DD or *ACE* I/D genotype have higher circulating levels of angiotensin-converting enzyme (ACE) which leads to activation of the renin-angiotensin-aldosterone system and myocardial fibrosis. Patients with *ACE* DD *or ACE* I/D genotype had a higher percentage of failure of response to AADs. Polymorphisms in adrenergic receptor genes have also been shown to modulate response to β-blockers. A study conducted on patients from the Vanderbilt AF Registry showed that patients with the Gly389 polymorphism, in the *ADRB1* gene, which encodes the β1 receptor, responded better to rate control (OR: 1.42; 95% CI: 1.00-2.03; $P < 0.05$).[86] G389R is an LOF variant that acts synergistically with β-blockers and augments their effect. A study in a Japanese cohort of 159 patients found that in patients with Gly389 polymorphism, the efficacy of flecainide was reduced when co-administered with β-blockers.[87] Another polymorphism in the *ADRB1* gene, Arg389Arg was investigated in a substudy of the BEST (β-Blocker Evaluation of Survival Trial), which determined that Arg389 homozygotes showed a better response to bucindolol treatment, with a 74% reduction in new-onset AF (HR: 0.26; 95% CI: 0.12-0.57).[88] However, the Arg389Arg polymorphism was not associated with a better outcome in a clinical trial comparing rhythm status in a heart failure cohort randomized to bucindolol or metoprolol.[89] β1 receptors are the primary target for bucindolol, but polymorphisms in the *ADRA2C* gene, which encodes the α_{2C}-adrenergic receptor located in the prejunctional sympathetic nerve terminal, also modulate the response to β-blockers. A 4-amino-acid deletion in positions 322-325 generates an LOF phenotype that is associated with an exaggerated response to bucindolol in heart failure.[90] The presence of more than 1 variant can have a synergistic effect in reducing the efficacy of β-blockers.

Genetic polymorphisms can also affect drug efficacy by modulating pharmacokinetics. The *CYP2D6* gene which encodes the cytochrome P450 enzyme is a key enzyme involved in the metabolism of AADs. Flecainide is a CYP2D6 substrate, and variants in *CYP2D6* are associated with delayed clearance of flecainide and require more frequent monitoring of serum levels.[90] A population pharmacokinetic study showed that there was an age-related decline in flecainide clearance in different genotype groups of *CYP2D6* (22.1% in heterozygous extensive metabolizers and 49.5% in heterozygous intermediate and poor metabolizers).[91,92] Variants in the promoter of the *SCN5A* gene termed Asian specific promoter HapB genotype are associated with slowed conduction and various arrhythmias.[93] A study compared the efficacy of flecainide between the HapA and HapB genotypes in a cohort of 146 patients with supraventricular arrhythmias and determined that HapB carriers can achieve therapeutic efficacy at lower doses of flecainide (42.9% vs 68.8%; $P = 0.02$).[94]

Parvez et al[95] reported that 3 AF loci on chr4q25, chr16q22, and chr1q21 modulated response to AADs, and found that a common SNP (rs10033464) in the chr4q25 locus not only predicted response to AADs, but also demonstrated a differential response to class I vs class III AADs. Patients with this SNP had almost 4-fold higher odds of maintaining sinus rhythm when prescribed a class I AAD. Although its results were promising, the study was limited to whites of European descent. A large clinical trial in a multiethnic cohort is in progress to determine genetic modulators of response to class I vs class III AADs (NCT02347111). **Table 2** summarizes the variants modulating drug responses in the management of AF.

GENOTYPIC DIFFERENCES IN RESPONSE TO CATHETER ABLATION. Rhythm control therapy improves AF-related symptoms and maintenance of sinus rhythm.[96] The CABANA (Catheter Ablation vs Antiarrhythmic Drug Therapy for Atrial Fibrillation) trial showed that catheter ablation has higher efficacy in maintaining sinus rhythm compared with AADs.[97] However, recurrence after ablation is common and often requires repeated ablation. An improved understanding of the underlying atrial substrate may enable the selection of patients most likely to respond to ablation.[98] Although cross-sectional studies

TABLE 2 Genetic Modifiers of Response to AADs

Genetic Determinant	Modulation of Response to AADs	First Author
ACE	*ACE* DD or *ACE* I/D genotype were significant predictors of failure of response to AADs (OR: 2.25; 95% CI: 1.05-4.80; $P = 0.04$)	Darbar et al[85]
ARDB1	Gly389 polymorphism is associated with a better response to rate control (OR: 1.42; 95% CI: 1.00 to 2.03; $P < 0.05$)	Parvez et al[86]
	Gly389 reduced the efficacy of flecainide when co-administered with β-blockers ($P = 0.001$)	Doki et al[87]
	Arg389Arg showed a better response to bucindolol (HR: 0.26; 95% CI: 0.12-0.57)	Aleong et al[88]
ADRA2C	A 4-amino-acid loss-of-function deletion is associated with an exaggerated response to bucindolol in heart failure ($P = 0.012$)	O'Connor et al[90]
CYP2D6	Variants in CYP2D6 decrease the clearance of flecainide and require more frequent monitoring (22.1% in heterozygous extensive metabolizers, 49.5% in heterozygous intermediate and poor metabolizers)	Doki et al[91]
SCN5A Haplotype B	Haplotype B genotype is associated with higher therapeutic efficacy at lower doses of flecainide (42.9% vs 68.8%; $P = 0.022$)	Doki et al[94]
4q25 (rs2200733, rs10033464)	rs2200733, rs10033464 predicts recurrence of AF (OR: 3.27; 95% CI: 1.7-6.0; $P < 0.001$) and better response to AADs, and showed a differential response to class I vs class III AADs (OR: 10.0; 95% CI: 1.03-97.5; $P < 0.05$)	Parvez et al[95]

suggest that chr4q25 SNPs predict postablation AF recurrence,[99] the findings need to be confirmed in a prospective and randomized clinical trial before a genotype-based catheter ablation approach is considered. The differences in response to catheter ablation are most likely due to interindividual differences in AF mechanisms. An improved understanding of the myocardial substrate for AF will guide the ablation procedure, patient monitoring, and postablation follow-up.

ATRIAL-SPECIFIC AADs. Pharmacologic approaches for AF are aimed at inhibiting ectopic activity and preventing reentry by prolonging the APD. Class I AADs inhibit the I_{Na}, decrease conduction velocity, and suppress ectopic beats, whereas class III AADs inhibit potassium channels, prolong the APD, and prevent reentry.[99] However, a significant challenge with currently available AADs is their proarrhythmic side-effects.[100,101] A decrease in conduction velocity by class I AADs may promote r-eentry, especially in the presence of structural remodeling.[99] On the other hand, excessive prolongation of the APD by class III AADs in ventricles can lead to EADs and result in dangerous ventricular arrhythmias such as torsades de pointes. Atrial-specific drugs that target ion channels specifically expressed in the atria could avert the ventricular proarrhythmic effects. This idea has led to many preclinical investigations for atrial-selective therapy, which have shown promising results. However, none have shown any benefit in clinical trials thus far.[99] The failure of the initial clinical trials for inhibitors of atrial-selective currents acetylcholine-activated inwardly rectifying potassium current (I_{Kach}) and ultrarapid-activating delayed rectifier potassium channel (I_{Kur}) along with the success of ablation therapy in symptom reduction after ablation reduced the enthusiasm for developing atrial-selective pharmacologic therapy. However, AADs continue to be commonly used for AF management and may be essential when there are contraindications to ablation therapy. **Table 3** summarizes trials investigating atrial-specific therapy.

In permanent AF, shortening of the APD that leads to reentry[102] is mainly brought about by increasing potassium currents.[103] Potassium channels are the major players in determining the APD and waveform. Calcium-activated potassium channels of small conductance (SK1-3) are an emerging atrial-specific target. They are encoded by the *KCNN1-3* genes and conduct the I_{KCa} current, which contributes to AP repolarization and is increased in permanent AF.[104] However, the expression of SK channels increases in the ventricles in heart failure patients, which would result in the loss of atrial selectivity.[105] An SK channel inhibitor, AP30663, is currently in a phase 2 clinical trial for the conversion of AF (NCT04571385).[106] *TWIK*-related acid-sensitive potassium channels (TASK-1) encoded by *KCNK3* conduct the I_{K2P} current and are responsible for background currents and stabilization of resting membrane potential.[107] TASK-1 is up-regulated in patients with permanent AF and is predominantly expressed in the atria. Pharmacologic inhibition of TASK-1 leads to APD prolongation.[108] A respiratory stimulant, doxapram, that has TASK-1 inhibitor properties is being investigated for AF conversion in the DOCTOS (Doxapram Conversion To Sinus Rhythm; EudraCT 2018-002979-17) trial. The acetylcholine-activated inwardly rectifying potassium channel and the ultrarapid-activating delayed rectifier channel are expressed in the atria. However, clinical trials of I_{KAch} and I_{Kur} inhibitors have not demonstrated any significant benefit in reducing AF burden.[109,110]

TABLE 3 Atrial Selective Drug Targets for AF

Current	Gene	Channel Subunit	Progress in Development	First Author
I_{KCa}	*KCNN1 (1-2)*	SK (1-3)	An SK channel inhibitor, AP30663, is currently in a phase 2 clinical trial for the conversion of AF (NCT04571385)	Gal et al[106]
I_{KP}	*KCNK3*	TASK-1	A respiratory stimulant, doxapram, that has TASK-1 inhibitor properties is being investigated for AF conversion in the Doxapram Conversion To Sinus Rhythm (DOCTOS) trial	EudraCT 2018-002979-17
I_{Na}	*SCN5A*	Nav1.5	Ranolazine and vernakalant are nonselective blockers that show frequency- and state-dependent binding kinetics	McIntyre et al[117] and Guerra et al[118]
I_{Kur}	*KCNA5*	Kv1.5	S66913 Did not decrease the AF burden in the DIAGRAF-IKUR clinical trial, potentially because I_{Kur} Is decreased in AF	Camm et al[109]
I_{KAch}	*KCNJ3/KCNJ5*	Kir3.1/Kir3.4	BMS 914392 did not decrease AF burden in a clinical trial in patients with paroxysmal AF	Podd et al[110]

In permanent AF there is constitutive activity of I_{KACh}.[111,112] Several I_{KAch} blockers have shown to be effective in preclinical models but have failed to reduce AF burden in clinical trials. Some of the currently used AADs have I_{KAch}-blocking properties, such as dronedarone, amiodarone, propafenone, and flecainide; however, they block other ion channels as well.[113-115] Although the initial trials have failed or were aborted early on, because Kir3.1/3.4 channels are primarily expressed in atria there is potential for developing I_{KAch}-specific inhibitors for AF. Kv1.5 potassium channel encoded by *KCNA5* conducts I_{Kur}. Studies have shown an increase in this current at high pacing rates. One potential reason why I_{Kur} inhibitors have failed to show any benefit in clinical trials is that I_{Kur} is decreased in AF.[109,116] Other potassium channels that contribute to APD shortening include increased basal inward-rectifier potassium current (I_{K1}), I_{Ks}, and I_{to}.

STATE-DEPENDENT INHIBITION OF SODIUM CHANNELS. EP differences between the atria and ventricles promote the atrial selective binding of sodium channel blockers. The more depolarized RMP in atria and high atrial rates in AF favor the binding of sodium channel blockers in the atria such as Ranolazaine and Vernakalant. Vernakalant also inhibits the atrial-specific I_{KAch} and I_{Kur}.[117,118]

TRANSLATION OF GENETIC DISCOVERIES TO THE CLINICAL CARE OF PATIENTS

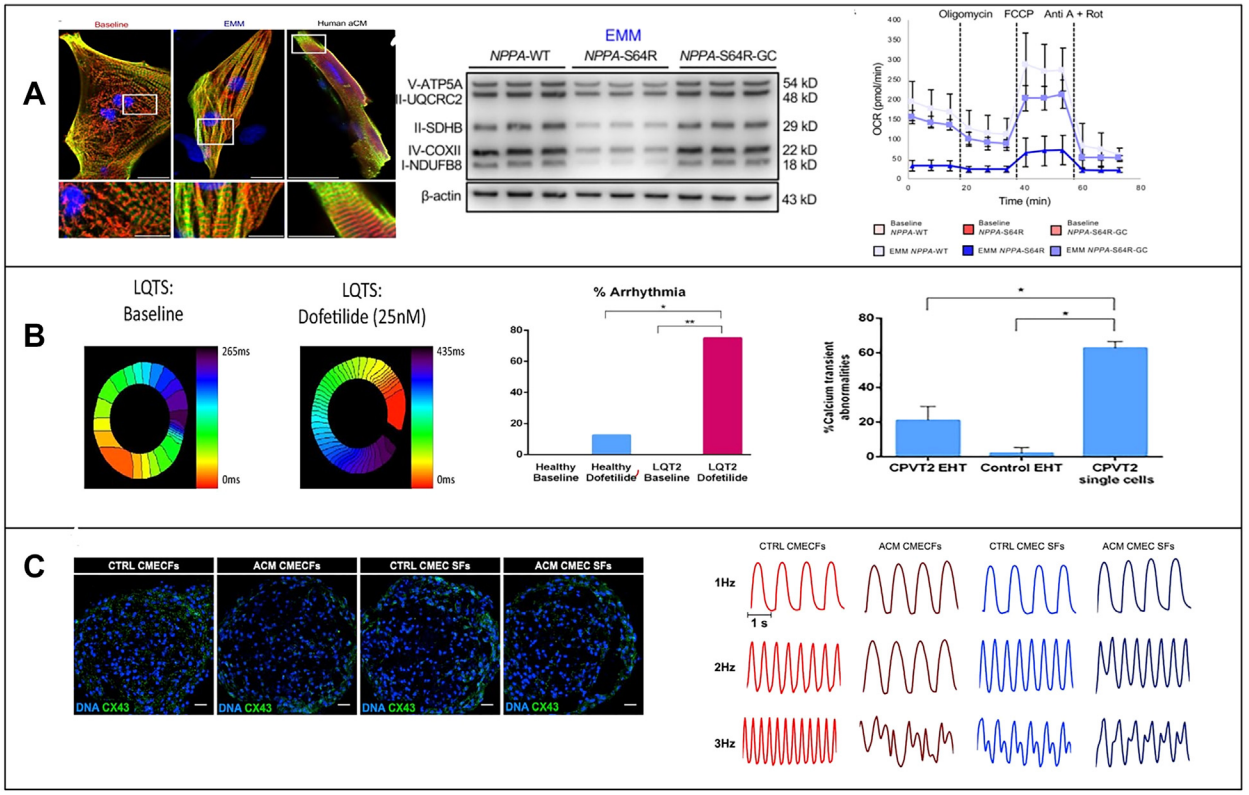
HIGH-THROUGHPUT ASSAYS FOR FUNCTIONAL ASSESSMENT OF VUS. According to the American College of Medical Genetics and Genomics, variants are classified as pathogenic, likely pathogenic, benign, or likely benign.[119] Variants that have insufficient evidence for clinical classification are labeled as VUS. VUS can account for more than 50% of known variants for a certain gene.[7] Whereas in vitro functional studies can effectively validate pathogenic and benign variants, assays lag for functional validation of VUS. Recently, a few groups have developed high-throughput assays for screening thousands of variants using multiplexed assays for variant evaluation (MAVE).

Muhammad et al[7] screened 2,592 VUS in *KCNE1* with the use of a MAVE that assessed potassium flux and cell surface expression as a readout. Their results were concordant with EP and computational analysis. Their approach could potentially be used to reclassify VUS.[7] Anderson et al[8] recently performed a high-throughput analysis of *LMNA* VUS and showed that most pathogenic *LMNA* variants are prone to aggregation in the nucleoplasm. They overexpressed 178 variants in myoblasts and HEK293 cells and performed aggregation analysis. They examined the phenotype for a small subset of variants in hiPSC-CMs as well.

BIOENGINEERING ADVANCES IN PATIENT-SPECIFIC hiPSC-DERIVED CARDIAC MODELS. Bioengineering advances in hiPSC-based disease modeling have given rise to an array of 2-dimensional (2D) and 3-dimensional (3D) cardiac models, such as micropatterned co-cultures, spheroids, organoids, EHT, microphysiologic heart-on-a-chip, and multiorgan-on-chip platforms for testing pharmacologic responses and understanding disease mechanisms (**Figure 2**).[120,121] hiPSC-derived cardiac models with nonmyocyte cells of the heart not only better recapitulate human cardiac physiology, but also are important to study of the effects of genetic variants on noncardiomyocytes that may contribute to disease pathogenesis.

A recent micropatterned co-culture platform of hiPSC-CMs and cardiac fibroblasts significantly improved the structural, metabolic, EP, and contractile kinetics compared with random monocultures.[122] Patient-specific 3D models of EHT for modeling long QT syndrome elicited arrhythmias only in response to QT prolongation agents, whereas 2D models showed frequent arrhythmias (**Figure 2B**).[9] A microtissue model of arrhythmogenic cardiomyopathy consisting of cardiomyocytes, endothelial cells, and cardiac

FIGURE 2 From 2-Dimensional Monolayers to 3-Dimensional Organoids in Disease Modeling and Drug Development for Cardiac Arrhythmias



(A) An electrometabolic maturation (EMM) approach reveals a mitochondrial defect in human-induced pluripotent stem cell-derived atrial cardiomyocytes (hiPSC-aCMs) as an underlying mechanism of atrial fibrillation due to an *NPPA* mutation (reproduced from Ly et al[77] with permission). Immunofluorescence staining for cardiac troponin T and actinin shows sarcomeric organization in EMM hiPSC-aCMs similar to that of human atrial tissue. Western blotting of mitochondrial oxidative phosphorylation proteins shows decreased expression only in the EMM hiPSC-aCMs with the *NPPA-S64R* variant. EMM *NPPA* S64R hiPSC-aCMs have reduced respiration rate, indicating the necessity of using matured hiPSC-aCMs. (B) Activation maps of ring-shaped engineered heart tissues (EHTs) from patients with long QT syndrome (LQTS) showing reentry on administration of 25 nmol/L dofetilide. LQTS EHT showed less frequent arrhythmias on administration of dofetilide compared with 2-dimensional monolayers owing to differences in drug diffusion kinetics (reproduced from Goldfracht et al[9] with permission). (C) A microtissue model of arrhythmogenic cardiomyopathy consisting of cardiomyocytes, endothelial cells, and cardiac fibroblasts reveals decreased expression of connexin-43 and arrhythmic contraction patterns but no change in microtissues with SF (reprinted from Giacomelli et al[123] with permission). CF = cardiac fibroblast; CPVT = catecholaminergic polymorphic ventricular tachycardia; EC = endothelial cell; EHT = engineered heart tissue; SF = skin fibroblast.

fibroblasts revealed decreased expression of CX43 in atrial fibroblasts and arrhythmic contraction patterns (**Figure 2C**).[123]

One of the main limitations of hiPSC-derived cardiac models is their immature phenotype, which resembles fetal cardiomyocytes more than adult cardiomyocytes. This remains a challenge in the reliable prediction of drug responses and characterization of genetic variants. A maturation strategy developed by Ly et al,[77] comprising electrical stimulation and metabolic conditioning, markedly improved the structural and EP characteristics of hiPSC-aCMs and revealed a mitochondrial defect due to a variant in the *NPPA* gene encoding A-type natriuretic peptide. The EP studies showed a shortening of APD and an increase in I_{Ks}. That study highlighted targeting I_{Ks} as a potential therapy for AF (**Figure 2A**). We and others have shown that the shortening of APD is due to an increase in I_{Ks},[22] but the partial contribution of I_{Ks} to atrial repolarization reserve should also be recognized. Furthermore, because I_{Ks} is not atrial specific, blocking I_{Ks} may have torsadogenic effects.[124,125]

PRS FOR DETERMINING AF RISK. The large number of common genetic variants associated with AF identified by GWAS can be used to determine genetic susceptibility to AF. A PRS, computed by the weighted sum of AF-associated SNPs and their effect size, provides an estimate of the cumulative genetic risk for AF. Early studies performed in European and

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 7, 2024
JULY 2024:918–934

Japanese populations found that variants at the chromosome 4q25 locus conferred a 5-fold increase in the risk of AF.[126] The AFGen Consortium found that while PRS for AF were associated with AF and stroke beyond clinical risk factors, they were not incremental in predicting the risk of arrhythmia.[127] Recently, a PRS for AF, using 6.6 million SNPs in the UK Biobank, reported that 6.1% of the general population was at a 3-fold increased risk for AF.[128] Although PRS are independent predictors of AF risk, the integration of clinical risk factors and biomarkers may further refine risk assessment. This was recently shown by Marston et al,[129] with a 6.7-million-SNP PRS independently predicting AF risk, and combining the PRS with clinical risk factors and N-terminal pro-B-type natriuretic peptide providing superior risk prediction.

Calculating a PRS predicts not only AF risk and adverse outcomes such as stroke, but also response to AAD and ablation therapy.[5] Identifying AF as a cause of ischemic stroke is important to initiate anticoagulant therapy to prevent recurrent strokes. Using AF PRS to predict the risk of stroke due to AF can be incremental over cardiac rhythm monitoring. Pulit et al[130] determined that the genetic risk score for AF was associated with cardioembolic stroke independently from clinical risk factors. A genetic risk score for stroke using 32 SNPs predicted stroke independently from traditional risk factors. Those with AF in that study with a low CHA_2DS_2-VASc score but a high genetic risk score had a risk of stroke similar to those with a higher CHA_2DS_2-VASc score.[131] The addition of clinical risk to PRSs for AF can also discriminate between cardioembolic and noncardioembolic causes of stroke.[132] A combination of AF PRS and clinical risk is emerging as a promising strategy to reduce morbidity due to AF-associated stroke.

Despite the advances in AF genomics, there are some limitations and challenges that need to be overcome before a genome-informed PRS can be implemented into clinical decision making. First, GWAS-derived PRSs are largely based on individuals of European descent. Genomic differences between ancestries with differing genetic variant frequencies and phenotype severity warrant GWAS to be performed in diverse populations.[5] Diversification of GWAS can also mitigate differences due to socioeconomic disparities within populations, which may affect AF-associated outcomes and access to health care. Another limitation is that there are no guidelines that define the threshold for initiation of preventive therapy based on genome-informed PRS for AF. We need to validate if AF PRS can predict subclinical AF and identify those at the highest risk for stroke and heart failure. Implementation of strategies for risk stratification may not only improve AF diagnosis and management, but also prevent AF-associated morbidities.

INTEGRATION OF AF GENETICS IN CLINICAL CARE. Current guidelines do not recommend genetic testing in AF. With the decreasing cost of genetic testing, it may not be cost-prohibitive to be used widely. However, several questions remain to be answered regarding the integration of AF genetics in clinical care. Specifically, who should be offered genetic testing: Should it be offered broadly or to a selected group of patients most likely to have a genetic basis for their disease? An increasing body of evidence supports the utility of candidate gene screening in patients with early-onset AF with a strong family history of cardiac disease.[11,133] Early detection can prevent the onset of structural changes and associated comorbidities. Genetic testing not only will facilitate early detection of AF in the individual, but also may have important implications for personalized therapy. Equally important is cascade screening of first-degree relatives who may be at risk of developing phenotypes other than AF, such as dilated cardiomyopathy and neuromuscular disease. For the broader population at risk for AF, while emerging studies support the utility of AF PRS in clinical risk stratification,[129,132] further studies are needed to validate whether a genome-informed AF PRS is superior to traditional risk stratification tools in predicting AF risk and AF-associated adverse outcomes.

CONCLUSIONS AND PERSPECTIVE

AF is a complex disease with significant clinical and genetic heterogeneity. Improved understanding of the myocardial substrate for AF and genotype-guided prospective trials are urgently needed for translating genetic discoveries to the bedside care of patients. Although there have been significant advances in ablation and device therapy for AF owing in part to the AF epidemic, AADs will remain important for therapy. Identification of variant-specific mechanisms and new molecular pathways and targets will broaden the scope of AADs, which currently target ion channels only. Functional validation of VUS and limitations of existing disease models are challenges in the translation of genetic discoveries to the bedside care of patients. These challenges are being addressed by high-throughput multiplexed assays for the reclassification of VUS and novel bioengineering strategies to develop hiPSC-derived cardiac models with increasing complexity to reliably recapitulate AF pathophysiology and predict drug responses. These

translational advances have the potential to develop personalized therapy for this common and morbid arrhythmia.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Drs Darbar and Khetani are supported by National Institutes of Health (NIH) grant R01 HL150586. Dr Darbar is supported by NIH grants R01 HL148444 T32 HL139439 and Veterans Administration Merit Review 1I01BX004268. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Dawood Darbar, Division of Cardiology, University of Illinois, 840 South Wood Street, 920S (MC 715), Chicago, Illinois 60612, USA. E-mail: darbar@uic.edu.

REFERENCES

1. Benjamin EJ, Wolf PA, d'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation*. 1998;98:946–952.

2. Miyasaka Y, Barnes ME, Gersh BJ, et al. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. *Circulation*. 2006;114:119–125.

3. Duytschaever M, Demolder A, Phlips T, et al. Pulmonary vein isolation with vs without continued antiarrhythmic Drug treatment in subjects with recurrent atrial fibrillation (POWDER AF): results from a multicentre randomized trial. *Eur Heart J*. 2018;39:1429–1437.

4. Darbar D, Roden DM. Genetic mechanisms of atrial fibrillation: impact on response to treatment. *Nat Rev Cardiol*. 2013;10:317–329.

5. Kany S, Reissmann B, Metzner A, Kirchhof P, Darbar D, Schnabel RB. Genetics of atrial fibrillation-practical applications for clinical management: if not now, when and how? *Cardiovasc Res*. 2021;117:1718–1731.

6. Kavousi M, Ellinor PT. Polygenic risk scores for prediction of atrial fibrillation. *Neth Heart J*. 2023;31:1–2.

7. Muhammad A, Calandranis ME, Li B, et al. High-throughput functional mapping of variants in an arrhythmia gene, KCNE1, reveals novel biology. *bioRxiv*. 2023:04.28.538612.

8. Anderson CL, Langer ER, Routes TC, et al. Most myopathic lamin variants aggregate: a functional genomics approach for assessing variants of uncertain significance. *NPJ Genom Med*. 2021;6:103.

9. Goldfracht I, Efraim Y, Shinnawi R, et al. Engineered heart tissue models from hiPSC-derived cardiomyocytes and cardiac ECM for disease modeling and drug testing applications. *Acta Biomater*. 2019;92:145–159.

10. Cho S, Discher DE, Leong KW, Vunjak-Novakovic G, Wu JC. Challenges and opportunities for the next generation of cardiovascular tissue engineering. *Nat Methods*. 2022;19:1064–1071.

11. Yoneda ZT, Anderson KC, Quintana JA, et al. Early-onset atrial fibrillation and the prevalence of rare variants in cardiomyopathy and arrhythmia genes. *JAMA Cardiol*. 2021;6:1371–1379.

12. Remme CA, Bezzina CR. Review: sodium channel (dys)function and cardiac arrhythmias. *Cardiovasc Ther*. 2010;28:287–294.

13. Priest BT, McDermott JS. Cardiac ion channels. *Channels (Austin)*. 2015;9:352–359.

14. Li Q, Huang H, Liu G, et al. A gain-of-function mutation of Nav1.5 in atrial fibrillation enhances cellular excitability and lowers the threshold for action potential firing. *Biochem Biophys Res Commun*. 2009;380:132–137.

15. Savio-Galimberti E, Weeke P, Muhammad R, et al. SCN10A/Nav1.8 modulation of peak and late sodium currents in patients with early-onset atrial fibrillation. *Cardiovasc Res*. 2014;104:355–363.

16. Van den Boogaard M, Smemo S, Burnicka-Turek O, et al. A common genetic variant within SCN10A modulates cardiac SCN5A expression. *J Clin Invest*. 2014;124:1844–1852.

17. Hong L, Zhang M, Ly OT, et al. Human induced pluripotent stem cell-derived atrial cardiomyocytes carrying an SCN5A mutation identify nitric oxide signaling as a mediator of atrial fibrillation. *Stem Cell Rep*. 2021;16:1542–1554.

18. Koval OM, Snyder JS, Wolf RM, et al. Ca^{2+}/calmodulin-dependent protein kinase II-based regulation of voltage-gated Na^{+} channel in cardiac disease. *Circulation*. 2012;126:2084–2092.

19. Wada Y, Yang T, Shaffer CM, et al. Common ancestry-specific ion channel variants predispose to drug-induced arrhythmias. *Circulation*. 2022;145:299–308.

20. Olson TM, Alekseev AE, Liu XK, et al. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. *Hum Mol Genet*. 2006;15:2185–2191.

21. Christophersen IE, Olesen MS, Liang B, et al. Genetic variation in KCNA5: impact on the atrial-specific potassium current IKur in patients with lone atrial fibrillation. *Eur Heart J*. 2013;34:1517–1525.

22. Chen YH, Xu SJ, Bendahhou S, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science*. 2003;299:251–254.

23. Mahida S, Lubitz SA, Rienstra M, Milan DJ, Ellinor PT. Monogenic atrial fibrillation as pathophysiological paradigms. *Cardiovasc Res*. 2011;89:692–700.

24. Brugada R, Tapscott T, Czernuszewicz GZ, et al. Identification of a genetic locus for familial atrial fibrillation. *N Engl J Med*. 1997;336:905–911.

25. Feghaly J, Zakka P, London B, MacRae CA, Refaat MM. Genetics of atrial fibrillation. *J Am Heart Assoc*. 2018;7:e009884.

26. Ehrlich JR. Inward rectifier potassium currents as a target for atrial fibrillation therapy. *J Cardiovasc Pharmacol*. 2008;52:129–135.

27. Pandit SV, Berenfeld O, Anumonwo JM, et al. Ionic determinants of functional reentry in a 2-D model of human atrial cells during simulated chronic atrial fibrillation. *Biophys J*. 2005;88:3806–3821.

28. Ellinor PT, Lunetta KL, Glazer NL, et al. Common variants in KCNN3 are associated with lone atrial fibrillation. *Nat Genet*. 2010;42:240–244.

29. Mancarella S, Yue Y, Karnabi E, Qu Y, El-Sherif N, Boutjdir M. Impaired Ca^{2+} homeostasis is associated with atrial fibrillation in the α1D L-type Ca^{2+} channel KO mouse. *Am J Physiol Heart Circ Physiol*. 2008;295:H2017–H2024.

30. Srivastava U, Aromolaran AS, Fabris F, et al. Novel function of α1D L-type calcium channel in the atria. *Biochem Biophys Res Commun*. 2017;482:771–776.

31. Lu L, Sirish P, Zhang Z, et al. Regulation of gene transcription by voltage-gated L-type calcium channel, Cav1.3. *J Biol Chem*. 2015;290:4663–4676.

32. Zhabyeyev P, Hiess F, Wang R, Liu Y, Wayne Chen SR, Oudit GY. S4153R is a gain-of-function mutation in the cardiac Ca^{2+} release channel ryanodine receptor associated with catecholaminergic polymorphic ventricular tachycardia and paroxysmal atrial fibrillation. *Can J Cardiol*. 2013;29:993–996.

33. Gollob MH, Jones DL, Krahn AD, et al. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. *N Engl J Med*. 2006;354:2677–2688.

34. Christophersen IE, Holmegard HN, Jabbari J, et al. Rare variants in GJA5 are associated with early-onset lone atrial fibrillation. *Can J Cardiol*. 2013;29:111–116.

35. Lubitz SA, Ellinor PT. A common connexion between gap junctions, single nucleotide polymorphisms, and atrial fibrillation? *Can J Cardiol*. 2013;29:3–5.

36. Noureldin M, Chen H, Bai D. Functional characterization of novel atrial fibrillation-linked GJA5 (Cx40) mutants. *Int J Mol Sci*. 2018;19:977.

37. Gudbjartsson DF, Arnar DO, Helgadottir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature*. 2007;448(7151):353–357.

38. Zhang M, Hill MC, Kadow ZA, et al. Long-range Pitx2c enhancer-promoter interactions prevent predisposition to atrial fibrillation. *Proc Natl Acad Sci U S A*. 2019;116:22692–22698.

39. Tao Y, Zhang M, Li L, et al. Pitx2, an atrial fibrillation predisposition gene, directly regulates

ion transport and intercalated disc genes. *Circ Cardiovasc Genet*. 2014;7:23–32.

40. Clauss S, Kääb S. Is Pitx2 growing up? *Circ Cardiovasc Genet*. 2011;4:105–107.

41. Muller II, Melville DB, Tanwar V, et al. Functional modeling in zebrafish demonstrates that the atrial-fibrillation-associated gene GREM2 regulates cardiac laterality, cardiomyocyte differentiation, and atrial rhythm. *Dis Model Mech*. 2013;6:332–341.

42. Schulz C, Lemoine MD, Mearini G, et al. PITX2 knockout induces key findings of electrical remodeling as seen in persistent atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2023;16:e011602.

43. Kim K, Blackwell DJ, Yuen SL, et al. The selective RyR2 inhibitor ent-verticilide suppresses atrial fibrillation susceptibility caused by Pitx2 deficiency. *J Mol Cell Cardiol*. 2023;180:1–9.

44. Benjamin EJ, Rice KM, Arking DE, et al. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. *Nat Genet*. 2009;41:879–881.

45. Rubio-Alarcón M, Cámara-Checa A, Dago M, et al. Zfhx3 transcription factor represses the expression of SCN5A gene and decreases sodium current density (I_{Na}). *Int J Mol Sci*. 2021;22:13031.

46. Roselli C, Chaffin MD, Weng LC, et al. Multi-ethnic genome-wide association study for atrial fibrillation. *Nat Genet*. 2018;50:1225–1233.

47. Wang J, Sun Y-M, Yang Y-Q. Mutation spectrum of the GATA4 gene in patients with idiopathic atrial fibrillation. *Mol Biol Rep*. 2012;39:8127–8135.

48. Haïssaguerre M, Jaïs P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med*. 1998;339:659–666.

49. Chen J, Xu S, Li W, et al. Nkx2.5 insufficiency leads to atrial electrical remodeling through Wnt signaling in HL-1 cells. *Exp Ther Med*. 2019;18:4631–4636.

50. Shiratori H, Sakuma R, Watanabe M, et al. Two-step regulation of left-right asymmetric expression of Pitx2: initiation by nodal signaling and maintenance by Nkx2. *Mol Cell*. 2001;7:137–149.

51. Yang Y-Q, Wang M-Y, Zhang X-L, et al. GATA4 loss-of-function mutations in familial atrial fibrillation. *Clin Chim Acta*. 2011;412:1825–1830.

52. Ellinor PT, Lunetta KL, Albert CM, et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet*. 2012;44:670–675.

53. Lin H, Sinner MF, Brody JA, et al. Targeted sequencing in candidate genes for atrial fibrillation: the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) targeted sequencing study. *Heart Rhythm*. 2014;11:452–457.

54. Bergwerff M, Gittenberger-de Groot AC, Wisse LJ, et al. Loss of function of the Prx1 and Prx2 homeobox genes alters the architecture of the great elastic arteries and ductus arteriosus. *Virchows Arch*. 2000;436:12–19.

55. Hoogaars WM, Engel A, Brons JF, et al. Tbx3 controls the sinoatrial node gene program and imposes pacemaker function on the atria. *Genes Dev*. 2007;21:1098–1112.

56. Postma AV, Van de Meerakker JB, Mathijssen IB, et al. A gain-of-function TBX5 mutation is associated with atypical Holt-Oram syndrome and paroxysmal atrial fibrillation. *Circ Res*. 2008;102:1433–1442.

57. Hiroi Y, Kudoh S, Monzen K, et al. Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. *Nat Genet*. 2001;28:276–280.

58. Miyazawa K, Ito K, Ito M, et al. Cross-ancestry genome-wide analysis of atrial fibrillation unveils disease biology and enables cardioembolic risk prediction. *Nat Genet*. 2023;55:187–197.

59. Choi SH, Weng LC, Roselli C, et al. Association between titin loss-of-function variants and early-onset atrial fibrillation. *JAMA*. 2018;320:2354–2364.

60. Orr N, Arnaout R, Gula LJ, et al. A mutation in the atrial-specific myosin light chain gene (MYL4) causes familial atrial fibrillation. *Nat Commun*. 2016;7:11303.

61. Nattel S. Close connections between contraction and rhythm: a new genetic cause of atrial fibrillation/cardiomyopathy and what it can teach us. *Eur Heart J*. 2017;38:35–37.

62. Gudbjartsson DF, Holm H, Sulem P, et al. A frameshift deletion in the sarcomere gene MYL4 causes early-onset familial atrial fibrillation. *Eur Heart J*. 2017;38:27–34.

63. Goette A, Kalman JM, Aguinaga L, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Europace*. 2016;18:1455–1490.

64. Zhang Y, Lin Y, Zhang Y, et al. Familial atrial myopathy in a large multigenerational heart-hand syndrome pedigree carrying an LMNA missense variant in rod 2B domain (p.R335W). *Heart Rhythm*. 2022;19:466–475.

65. Tremblay-Gravel M, Ichimura K, Picard K, et al. Intrinsic atrial myopathy precedes left ventricular dysfunction and predicts atrial fibrillation in lamin A/C cardiomyopathy. *Circ Genom Precis Med*. 2023;16:e003480.

66. Frank D, Frey N. Cardiac Z-disc signaling network. *J Biol Chem*. 2011;286:9897–9904.

67. Ahlberg G, Refsgaard L, Lundegaard PR, et al. Rare truncating variants in the sarcomeric protein titin associated with familial and early-onset atrial fibrillation. *Nat Commun*. 2018;9:4316.

68. Schafer S, de Marvao A, Adami E, et al. Titin-truncating variants affect heart function in 8 disease cohorts and the general population. *Nat Genet*. 2017;49:46–53.

69. Wang TJ, Larson MG, Levy D, et al. Temporal relations of atrial fibrillation and congestive heart failure and their joint influence on mortality: the Framingham Heart Study. *Circulation*. 2003;107:2920–2925.

70. Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J Am Coll Cardiol*. 2012;60:705–715.

71. van Spaendonck-Zwarts KY, van Hessem L, Jongbloed JD, et al. Desmin-related myopathy. *Clin Genet*. 2011;80:354–366.

72. Schrickel JW, Stöckigt F, Krzyzak W, et al. Cardiac conduction disturbances and differential effects on atrial and ventricular electrophysiological properties in desmin deficient mice. *J Interv Card Electrophysiol*. 2010;28:71–80.

73. Smolina N, Khudiakov A, Knyazeva A, et al. Desmin mutations result in mitochondrial dysfunction regardless of their aggregation properties. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866:165745.

74. Thorolfsdottir RB, Sveinbjornsson G, Sulem P, et al. A missense variant in PLEC increases risk of atrial fibrillation. *J Am Coll Cardiol*. 2017;70:2157–2168.

75. Beavers DL, Wang W, Ather S, et al. Mutation E169K in junctophilin-2 causes atrial fibrillation due to impaired RyR2 stabilization. *J Am Coll Cardiol*. 2013;62:2010–2019.

76. Hodgson-Zingman DM, Karst ML, Zingman LV, et al. Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. *N Engl J Med*. 2008;359:158–165.

77. Ly OT, Chen H, Brown GE, et al. Mutant ANP induces mitochondrial and ion channel remodeling in a human iPSC-derived atrial fibrillation model. *JCI Insight*. 2022;7.

78. Captur G, Arbustini E, Bonne G, et al. Lamin and the heart. *Heart*. 2018;104:468.

79. Lazarte J, Jurgens SJ, Choi SH, et al. LMNA Variants and risk of adult-onset cardiac disease. *J Am Coll Cardiol*. 2022;80:50–59.

80. Pessente GD, Sacilotto L, Calil ZO, et al. Effect of occurrence of lamin A/C (LMNA) genetic variants in a cohort of 101 consecutive apparent "lone AF" patients: results and insights. *Front Cardiovasc Med*. 2022;9:823717.

81. Lee J, Termglinchan V, Diecke S, et al. Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy. *Nature*. 2019;572:335–340.

82. Salvarani N, Crasto S, Miragoli M, et al. The K219T-lamin mutation induces conduction defects through epigenetic inhibition of SCN5A in human cardiac laminopathy. *Nat Commun*. 2019;10:2267.

83. Zhang X, Chen S, Yoo S, et al. Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. *Cell*. 2008;135:1017–1027.

84. Oberti C, Wang L, Li L, et al. Genome-wide linkage scan identifies a novel genetic locus on chromosome 5p13 for neonatal atrial fibrillation associated with sudden death and variable cardiomyopathy. *Circulation*. 2004;110:3753–3759.

85. Darbar D, Motsinger AA, Ritchie MD, Gainer JV, Roden DM. Polymorphism modulates fibrillation. *Heart Rhythm*. 2007;4:743–749.

86. Parvez B, Chopra N, Rowan S, et al. A common β1-adrenergic receptor polymorphism predicts favorable response to rate-control therapy in atrial fibrillation. *J Am Coll Cardiol*. 2012;59:49–56.

87. Doki K, Sekiguchi Y, Kuga K, Aonuma K, Homma M. β1-Adrenergic receptor Arg389Gly polymorphism affects the antiarrhythmic efficacy of flecainide in patients with coadministration of β-blockers. *Pharmacogenet Genomics*. 2016;26:481–485.

88. Aleong RG, Sauer WH, Robertson AD, Liggett SB, Bristow MR. Adrenergic receptor polymorphisms and prevention of ventricular arrhythmias with bucindolol in patients with chronic heart failure. *Circ Arrhythm Electrophysiol*. 2013;6:137–143.

89. Piccini JP, Abraham WT, Dufton C, et al. Bucindolol for the maintenance of sinus rhythm in a genotype-defined HF population: the GENETIC-AF trial. *JACC Heart Fail*. 2019;7:586–598.

90. O'Connor CM, Fiuzat M, Carson PE, et al. Combinatorial pharmacogenetic interactions of bucindolol and β1, α2C adrenergic receptor polymorphisms. *PLoS One*. 2012;7:e44324.

91. Doki K, Homma M, Kuga K, Aonuma K, Kohda Y. CYP2D6 genotype affects age-related decline in flecainide clearance: a population pharmacokinetic analysis. *Pharmacogenet Genomics*. 2012;22:777–783.

92. Doki K. Use of pharmacogenetic information for therapeutic drug monitoring of an antiarrhythmic drug. Article in Japanese. *Yakugaku Zasshi*. 2018;138:1145–1150.

93. Yagihara N, Watanabe H, Barnett P, et al. Variants in the SCN5A promoter associated with various arrhythmia phenotypes. *J Am Heart Assoc*. 2016;5:e003644.

94. Doki K, Homma M, Kuga K, Aonuma K, Kohda Y. SCN5A promoter haplotype affects the therapeutic range for serum flecainide concentration in Asian patients. *Pharmacogenet Genomics*. 2013;23:349–354.

95. Parvez B, Vaglio J, Rowan S, et al. Symptomatic response to antiarrhythmic drug therapy is modulated by a common single nucleotide polymorphism in atrial fibrillation. *J Am Coll Cardiol*. 2012;60:539–545.

96. Willems S, Meyer C, de Bono J, et al. Cabins, castles, and constant hearts: rhythm control therapy in patients with atrial fibrillation. *Eur Heart J*. 2019;40:3793–3799c.

97. Packer DL, Mark DB, Robb RA, et al. Effect of Catheter Ablation vs Antiarrhythmic Drug Therapy on Mortality, Stroke, Bleeding, and Cardiac Arrest Among Patients With Atrial Fibrillation: The CABANA Randomized Clinical Trial. *JAMA*. 2019;321:1261–1274.

98. Reyat JS, Chua W, Cardoso VR, et al. Reduced left atrial cardiomyocyte PITX2 and elevated circulating BMP10 predict atrial fibrillation after ablation. *JCI Insight*. 2020;5:e139179.

99. Zimetbaum P. Antiarrhythmic drug therapy for atrial fibrillation. *Circulation*. 2012;125:381–389.

100. Heijman J, Hohnloser SH, Camm AJ. Antiarrhythmic drugs for atrial fibrillation: lessons from the past and opportunities for the future. *Europace*. 2021;23:ii14–ii22.

101. McCauley M, Vallabhajosyula S, Darbar D. Proarrhythmic and torsadogenic effects of potassium channel blockers in patients. *Card Electrophysiol Clin*. 2016;8:481–493.

102. Allessie M, Ausma J, Schotten U. Electrical, contractile and structural remodeling during atrial fibrillation. *Cardiovasc Res*. 2002;54:230–246.

103. Caballero R, de la Fuente MG, Gomez R, et al. In humans, chronic atrial fibrillation decreases the transient outward current and ultrarapid component of the delayed rectifier current differentially on each atria and increases the slow component of the delayed rectifier current in both. *J Am Coll Cardiol*. 2010;55:2346–2354.

104. Skibsbye L, Poulet C, Diness JG, et al. Small-conductance calcium-activated potassium (SK) channels contribute to action potential repolarization in human atria. *Cardiovasc Res*. 2014;103:156–167.

105. Darkow E, Nguyen TT, Stolina M, et al. Small conductance Ca^{2+}-activated K^+ (SK) channel mRNA expression in human atrial and ventricular tissue: comparison between donor, atrial fibrillation and heart failure tissue. *Front Physiol*. 2021;12:650964.

106. Gal P, Klaassen ES, Bergmann KR, et al. First clinical study with AP30663—a K_{Ca2} channel inhibitor in development for conversion of atrial fibrillation. *Clin Transl Sci*. 2020;13:1336–1344.

107. Burg S, Attali B. Targeting of potassium channels in cardiac arrhythmias. *Trends Pharmacol Sci*. 2021;42:491–506.

108. Schmidt C, Wiedmann F, Voigt N, et al. Upregulation of $K_{2P}3.1$ K+ current causes action potential shortening in patients with chronic atrial fibrillation. *Circulation*. 2015;132:82–92.

109. Camm AJ, Dorian P, Hohnloser SH, et al. A randomized, double-blind, placebo-controlled trial assessing the efficacy of S66913 in patients with paroxysmal atrial fibrillation. *Eur Heart J Cardiovasc Pharmacother*. 2019;5:21–28.

110. Podd SJ, Freemantle N, Furniss SS, Sulke N. First clinical trial of specific I_{KACh} blocker shows no reduction in atrial fibrillation burden in patients with paroxysmal atrial fibrillation: pacemaker assessment of BMS 914392 in patients with paroxysmal atrial fibrillation. *Europace*. 2015;18:340–346.

111. Voigt N, Trausch A, Knaut M, et al. Left-to-right atrial inward rectifier potassium current gradients in patients with paroxysmal versus chronic atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2010;3:472–480.

112. Dobrev D, Friedrich A, Voigt N, et al. The G protein-gated potassium current $I_{K,ACh}$ is constitutively active in patients with chronic atrial fibrillation. *Circulation*. 2005;112:3697–3706.

113. Guillemare E, Marion A, Nisato D, Gautier P. Inhibitory effects of dronedarone on muscarinic K^+ current in guinea pig atrial cells. *J Cardiovasc Pharmacol*. 2000;36:802–805.

114. Watanabe Y, Hara Y, Tamagawa M, Nakaya H. Inhibitory effect of amiodarone on the muscarinic acetylcholine receptor–operated potassium current in guinea pig atrial cells. *J Pharmacol Exp Ther*. 1996;279:617–624.

115. Voigt N, Rozmaritsa N, Trausch A, et al. Inhibition of $I_{K,ACh}$ current may contribute to clinical efficacy of class I and class III antiarrhythmic drugs in patients with atrial fibrillation. *Naunyn Schmiedebergs Arch Pharmacol*. 2010;381:251–259.

116. van Wagoner DR, Pond AL, McCarthy PM, Trimmer JS, Nerbonne JM. Outward K^+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. *Circ Res*. 1997;80:772–781.

117. McIntyre WF, Bhatnagar AK, Wang P, et al. Vernakalant for cardioversion of recent-onset atrial fibrillation: a systematic review and meta-analysis. *Eur Heart J*. 2018;39(Suppl 1):P2300.

118. Guerra F, Romandini A, Barbarossa A, Belardinelli L, Capucci A. Ranolazine for rhythm control in atrial fibrillation: a systematic review and meta-analysis. *Int J Cardiol*. 2017;227:284–291.

119. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424.

120. Mills RJ, Parker BL, Quaife-Ryan GA, et al. Drug screening in human PSC-cardiac organoids identifies pro-proliferative compounds acting via the mevalonate pathway. *Cell Stem Cell*. 2019;24:895–907.e6.

121. Archer CR, Sargeant R, Basak J, Pilling J, Barnes JR, Pointon A. Characterization and validation of a human 3D cardiac microtissue for the assessment of changes in cardiac pathology. *Sci Rep*. 2018;8:10160.

122. Brown G, Han Y, Michell A, et al. Engineered cocultures of iPSC-derived atrial cardiomyocytes and atrial fibroblasts for modeling atrial fibrillation. *Sci Adv*. 2024;10:eadg1222.

123. Giacomelli E, Meraviglia V, Campostrini G, et al. Human-iPSC-derived cardiac stromal cells enhance maturation in 3D cardiac microtissues and reveal noncardiomyocyte contributions to heart disease. *Cell Stem Cell*. 2020;26:862–879.e11.

124. Jost N, Virág L, Comtois P, et al. Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. *J Physiol*. 2013;591:4189–4206.

125. Varró A, Biliczki P, Iost N, et al. Theoretical possibilities for the development of novel antiarrhythmic drugs. *Curr Med Chem*. 2004;11:1–11.

126. Lubitz SA, Lunetta KL, Lin H, et al. Novel genetic markers associate with atrial fibrillation risk in Europeans and Japanese. *J Am Coll Cardiol*. 2014;63:1200–1210.

127. Lubitz SA, Yin X, Lin HJ, et al. Genetic risk prediction of atrial fibrillation. *Circulation*. 2017;135:1311–1320.

128. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. 2018;50:1219–1224.

129. Marston NA, Garfinkel AC, Kamanu FK, et al. A polygenic risk score predicts atrial fibrillation in cardiovascular disease. *Eur Heart J*. 2023;44:221–231.

130. Pulit SL, Weng LC, McArdle PF, et al. Atrial fibrillation genetic risk differentiates cardioembolic stroke from other stroke subtypes. *Neurol Genet*. 2018;4:e293.

131. Marston NA, Melloni GEM, Gurmu Y, et al. Genetic risk score to identify risk of venous thromboembolism in patients with cardiometabolic disease. *Circ Genom Precis Med*. 2021;14:e003006.

132. Weng LC, Khurshid S, Gunn S, et al. Clinical and genetic atrial fibrillation risk and discrimination of cardioembolic from non-cardioembolic stroke. *Stroke*. 2023;54:1777–1785.

133. Chalazan B, Freeth E, Mohajeri A, et al. Genetic testing in monogenic early-onset atrial fibrillation. *Eur J Hum Genet*. 2023;31:769–775.

KEY WORDS arrhythmia, genome-wide association study, induced pluripotent stem cell