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SCN5A variant that blocks fibroblast growth factor homologous factor regulation causes human arrhythmia

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Na_v channels are essential for metazoan membrane depolarization, and Na_v channel dysfunction is directly linked with epilepsy, ataxia, pain, arrhythmia, myotonia, and irritable bowel syndrome. Human Na_v channelopathies are primarily caused by variants that directly affect Na_v channel permeability or gating. However, a new class of human Na_v channelopathies has emerged based on channel variants that alter regulation by intracellular signaling or cytoskeletal proteins. Fibroblast growth factor homologous factors (FHFs) are a family of intracellular signaling proteins linked with Na_v channel regulation in neurons and myocytes. However, to date, there is surprisingly little evidence linking Na_v channel gene variants with FHFs and human disease. Here, we provide, to our knowledge, the first evidence that mutations in *SCN5A* (encodes primary cardiac Na_v channel $Na_v1.5$) that alter FHF binding result in human cardiovascular disease. We describe a five*generation kindred with a history of atrial and ventricular arrhythmias, cardiac arrest, and sudden cardiac death. Affected family members harbor a novel *SCN5A* variant resulting in p.H1849R. p.H1849R is localized in the central binding core on $Na_v1.5$ for FHFs. Consistent with these data, $Na_v1.5$ p.H1849R affected interaction with FHFs. Further, electrophysiological analysis identified $Na_v1.5$ p.H1849R as a gain-of-function for I_{Na} by altering steady-state inactivation and slowing the rate of $Na_v1.5$ inactivation. In line with these data and consistent with human cardiac phenotypes, myocytes expressing $Na_v1.5$ p.H1849R displayed prolonged action potential duration and arrhythmogenic afterdepolarizations. Together, these findings identify a previously unexplored mechanism for human Na_v channelopathy based on altered $Na_v1.5$ association with FHF proteins.

ion channel | channelopathy | atrial fibrillation | Nav1.5 | FHF

Encoded by 10 different genes, Na_v channel α-subunits regulate excitable membrane depolarization and are therefore central to metazoan physiology (1). Na_v channel function is critical for neuronal firing and communication (1, 2), cardiac excitation–contraction coupling (3), and skeletal and intestinal function (4, 5). The impact of Na_v channel function for human biology has been elegantly defined by nearly two decades of studies directly linking Na_v channel gene variants with human disease. To date, the field of human Na_v channelopathies has exploded to include wide spectrums of neurological [epilepsy (1), pain (6), ataxia (7)] and cardiovascular diseases (8) as well as myotonia congenital (9) and even irritable bowel syndrome (5). Though the majority of these diseases are based on gene variants in Na_v channel transmembrane segments that affect the channel pore or channel gating (10), a new paradigm for Na_v channelopathies has emerged based on variants that alter association of Na_v channels with essential regulatory proteins. To date, human Na_v channel gene variants linked with neurological and cardiovascular disease have not only provided new insight on the pathophysiology of excitable cell disease, but also identified and/or validated key in vivo Na_v channel regulatory pathways [syntrophin (11), ankyrin-G (12, 13), Na_v β1 (14), calmodulin (15), protein kinase A (16), and CaMKIIδ (17, 18)]. However, in many cases, whereas animal and cellular findings may strongly support the role of regulatory proteins for human Na_v channel function, human variants that may serve to validate the association have remained elusive, likely due to redundancy of signaling pathways or extreme severity of the disease.

Identified in the retina nearly two decades ago, fibroblast growth factor homologous factors (FHFs; FGF11–14) are a family of signaling proteins with key roles in ion channel regulation (19). Distinct from canonical FGFs that are secreted and bind to extracellular FGF receptors, FGF11–14 lack signal sequences and thus regulate intracellular targets. Currently, Na_v channels are the most characterized FHF target (20–22), and recent structural data mapped the FHF binding site to the Na_v channel C terminus (23, 24). Notably, FHFs display multiple roles in Na_v channel regulation, including expression, trafficking, and channel gating. However, each FHF appears to show unique regulatory roles for Na_v channel regulation that are cell type and Na_v channel isoform dependent. Though FHF signaling is complex, the roles of FHFs

Significance

Cardiovascular disease remains the leading cause of mortality in the United States, and cardiac arrhythmia underlies the majority of these deaths. Here, we report a new mechanism for congenital human cardiac arrhythmia due to defects in the regulation of the primary cardiac Na_v channel, $Na_v1.5$ (*SCN5A*), by a family of signaling molecules termed fibroblast growth factor homologous factors (FHFs). Individuals harboring *SCN5A* variants that affect $Na_v1.5$/FHF interactions display atrial and ventricular phenotypes, syncope, and sudden cardiac death. The human variant results in aberrant $Na_v1.5$ inactivation, causing prolonged action potential duration and afterdepolarizations in murine myocytes, thereby providing a rationale for the human arrhythmia.

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The authors declare no conflict of interest.

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in vertebrate physiology are clearly illustrated by dysfunction of FHFs in human disease. To date, FHF loci have been linked with spinocerebellar ataxia 27, X-linked mental retardation, and cardiac arrhythmia (25–27). In animals, FHF deficiency results in severe neurological phenotypes associated with altered Na_v channel function (28). Despite the overwhelming biochemical, functional, and in vivo animal data linking Na_v channels and FHF proteins, and in contrast to many other Na_v channel regulatory pathways, there are surprisingly little data linking human Na_v channel variants with FHFs in any disease.

Here we provide, to our knowledge, the first evidence that human Na_v channel gene variants that alter FHF binding result in potentially fatal human disease. We describe a five-generation kindred with a history of atrial and ventricular arrhythmias, cardiac arrest, and sudden cardiac death. Genetic testing revealed a *SCN5A* variant, resulting in p.H1849R, in affected family members. The identified *SCN5A* variant p.H1849R is novel and located at a site in the $Na_v1.5$ C-terminal domain identified to associate with FHFs. Notably, the human p.H1849R variant markedly altered interaction with FHFs, and functional analysis of the variant identified $Na_v1.5$ p.H1849R as a gain-of-function variant. Further, consistent with LQT3 phenotypes observed in the family, expression of this variant resulted in prolonged action potential duration and arrhythmogenic afterdepolarizations. Together, our findings define a previously unidentified mechanism for human Na_v channelopathies based on loss of $Na_v1.5$ association with FHF proteins and further confirm the critical link between these intracellular proteins and Na_v channels in excitable cells.

Results

$Na_v1.5$ Variant p.H1849R Associates with LQT, Atrial Fibrillation, Ventricular Tachycardia, and Sudden Cardiac Arrest. We identified a previously uncharacterized *SCN5A* variant associated with atrial fibrillation (AF), long QT, and sudden cardiac arrest. The proband is a 27-y-old male who suffered sudden cardiac arrest at work while moving boxes. Upon resuscitation with CPR and automated external defibrillator (AED) shock, initial ECG recordings presented episodes of AF, evident by the lack of P-waves and a varying R–R interval, accompanied by prevalent premature ventricular contractions (PVCs; Fig. 1*A*, white arrows). Subsequent interrogations revealed a prolonged QT interval, with a corrected QT (QT_c) up to 496 ms (sinus rhythm), augmented by ST segment changes and episodes of AF. Procainamide challenge of the proband was negative for Brugada (BrS) ECG pattern, and did not demonstrate J-point elevation or QRS prolongation. Serial echocardiograms were unremarkable in terms of ventricular function and atrial and ventricular dimensions. A diagnosis of LQT3 was made after serial rest ECGs disclosed persistent QTc prolongation. Based on phenotypes and witnessed arrest, the proband was implanted with dual chamber implantable cardioverter defibrillator (ICD). The ICD has appropriately fired multiple times in response to sustained ventricular tachycardia/fibrillation (Fig. 1*B*). Notably, several family members have presented cardiac abnormalities coincident with a family history of sudden cardiac death (SCD; Fig. 1*C*). Due to the high suspicion for primary arrhythmia disease and documented family history of SCD, the proband underwent targeted genetic testing for identified arrhythmia genes (*KCNQ1*, *KCNH2*, *SCN5A*, *ANK2*, *KCNE1*, *KCNE2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, and *SNTA1*). No known disease-associated variants were identified. However, secondary testing identified a heterozygous A > G nucleotide substitution in exon 28 of the *SCN5A* gene, producing a histidine-to-arginine change in $Na_v1.5$, the primary cardiac Na_v channel (Fig. 1 *D* and *E*; $Na_v1.5$ p.H1849R). The variant was not previously identified as disease causing, and has not been observed in the general population (minor allele frequency was 0.0 in ExAC and ClinVar). Follow-up ECGs consistently demonstrated periods of prolonged QTc, mild ST segment changes, AF, and PVCs.

Cascade screening showed that the proband's mother and sister harbored the $Na_v1.5$ p.H1849R variant (Fig. 1*C*). ECGs from the proband's mother show borderline prolonged QTc (QTc > 460 ms) with slight ST changes following exertion. Results from an implantable loop recorder (ILR) document several episodes of nonsustained AF with atrial and ventricular pacing, and a rate-dependent bundle branch block. Succeeding interrogations of the ILR demonstrated continued periods of AF, summating in a visit to the emergency room for a 2-h period of recorded symptomatic AF. Patient's sister reported a history of syncope, palpitations, and chest tightness following caffeine. Episodes of supraventricular

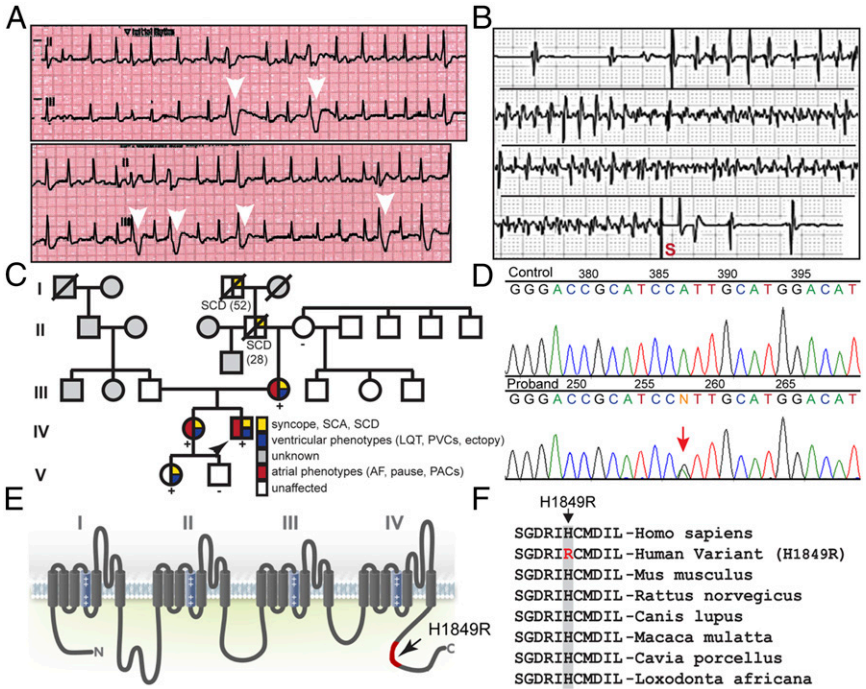

Fig. 1. Human $Na_v1.5$ H1849R variant associates with LQT and atrial fibrillation. (*A*) ECG records taken following resuscitation of a 27-y-old male experiencing sudden cardiac arrest. ECGs demonstrate atrial fibrillation and premature ventricular contractions (white arrows). (*B*) Malignant ventricular arrhythmia in proband requiring ICD discharge (note "S"). (*C*) Family pedigree of the proband (arrow) denoting members harboring the H1849R variant (+ symbol) and associated phenotypes. LQT, long QT, SCA, sudden cardiac arrest. (*D*) Chromatograms denoting the nucleotide change (A > G) resulting in a histidine-to-arginine change at amino acid position 1849 of $Na_v1.5$ in proband. (*E*) Membrane topology of $Na_v1.5$ protein denoting location the H1849R variant. (*F*) $Na_v1.5$ H1849 is highly conserved across species.

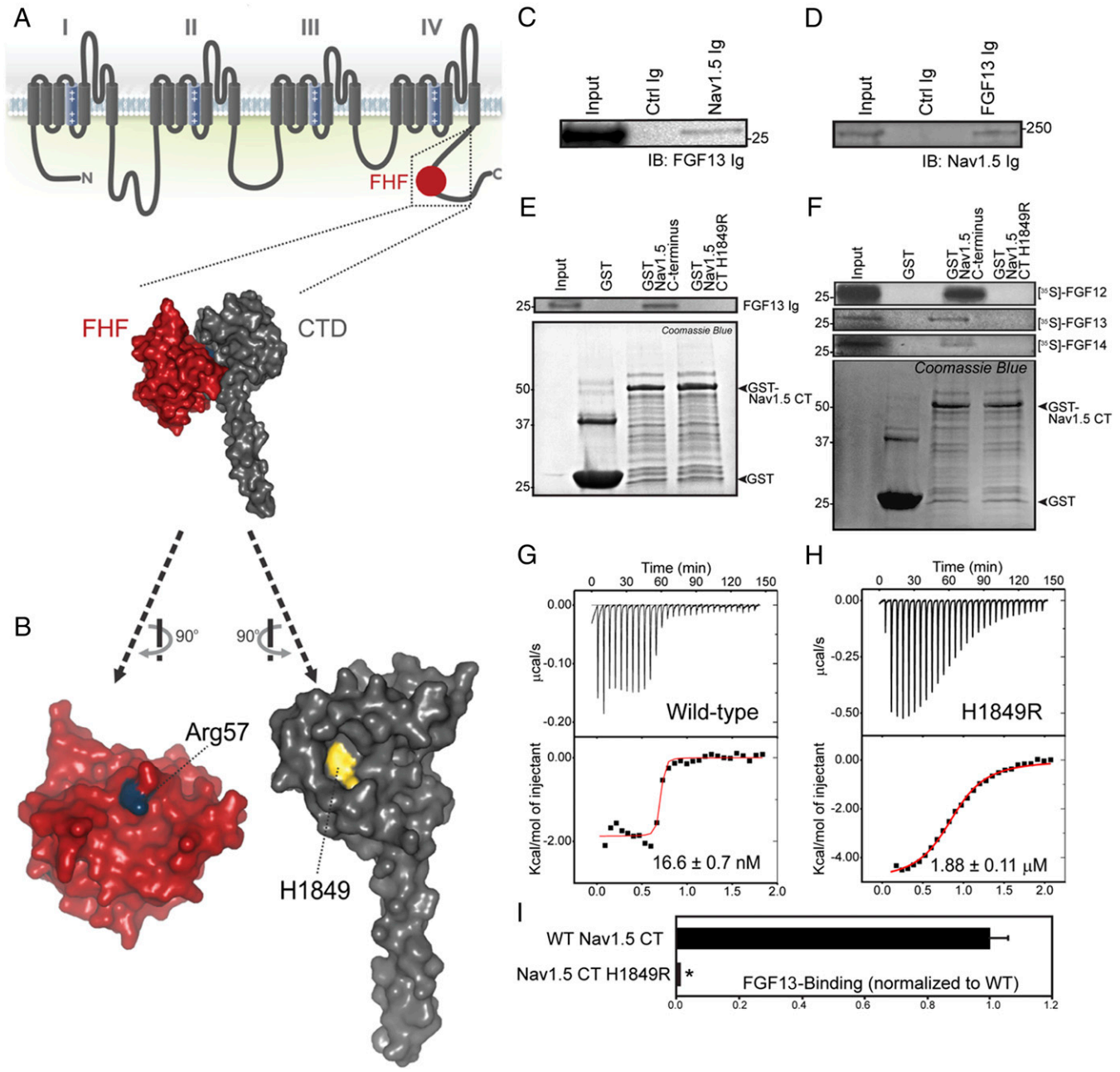

Fig. 2. Human $Na_v1.5$ p.H1849R variant blocks association with FHF proteins. (*A*) Schematic of $Na_v1.5$ denoting FHF binding domain in in red. (*B*) Interaction surfaces of FGF13 and $Na_v1.5$, denoting the critical Arg57 on FGF13 (blue) and its interaction pocket anchored by His1849 (yellow). (*C*) $Na_v1.5$ Ig coimmunoprecipitated FGF13 from detergent-soluble lysates of murine heart. (*D*) Conversely, FGF13 Ig coimmunoprecipitated $Na_v1.5$ from detergent-soluble lysates of murine heart. (*E*) Immobilized GST-$Na_v1.5$ C terminus (CT) associated with FGF13 from detergent-soluble lysates of murine hearts. In contrast, p.H1849R CT did not demonstrate significant binding. No interaction was observed between FGF13 and GST alone. (*F*) ^{35}S-labeled FGF12, 13, and 14 directly associate with GST-$Na_v1.5$ CT but not GST-$Na_v1.5$ p.H1849R. (*G* and *H*) Isothermal titration calorimetry data demonstrated a 10^2-fold decrease in binding affinity for FGF13 to the H1849R C-terminal domain (CTD) relative to WT CTD (K_d = 1.88 ± 0.11 µM and 16.6 ± 0.7 nM, respectively; $n = 3$, $P < 0.05$). (*I*) Qualitative rendering of binding affinity of $Na_v1.5$ CT H1849R relative to WT $Na_v1.5$ CT.

and ventricular ectopy were recorded on a Holter monitor, and subsequently an ILR was placed for future interrogation. After positive genetic screening in the proband's sister, further screening for the proband's niece and nephew was indicated, and consent was given for testing. Genetic screening of the niece (5 y) was positive for the p.H1849R variant, and follow-up was initiated for electrophysiology study testing. A diagnosis of LQT3 was made after serial-rest ECGs disclosed persistent QTc prolongation (QTc 471 ms). Intermittent late-peaking T waves were also appreciated. Other significant family history included the proband's maternal grandfather who had SCD in his sleep at the age of 28, and a maternal paternal great grandfather who had SCD in his sleep at the age of 52. No autopsies were performed on these individuals, and no DNA is available for evaluation.

$Na_v1.5$ p.H1849R Alters Association with FHFs. p.H1849 is located in the cytoplasmic C terminus of $Na_v1.5$ ($Na_v1.5$ CT) and conserved across species (Fig. 1 *E* and *F*). This specific residue was previously identified as forming part of the binding pocket for FHFs (23). A rendering of the interaction surfaces for FGF13 and the $Na_v1.5$

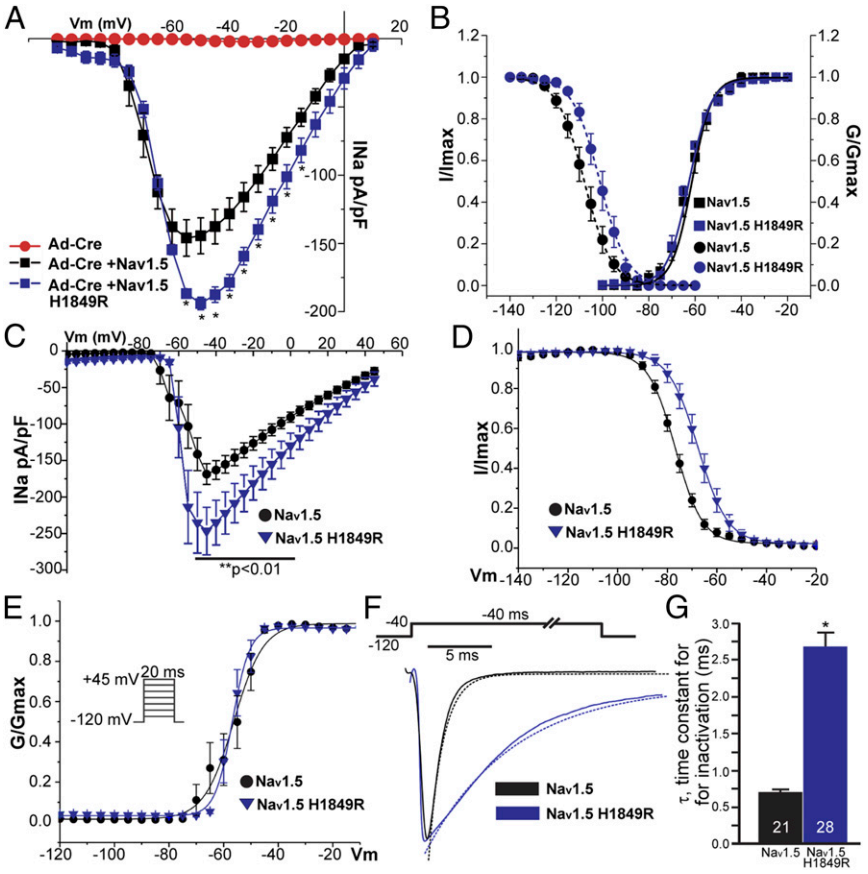

Fig. 3. Nav1.5 p.H1849R results in gain of function. (*A*) I_{Na} elicited from $Na_v1.5$ p.H1849R-expressing myocytes (blue, $n = 8$) displayed significantly larger I_{Na} density relative to cells expressing WT $Na_v1.5$ (black, $n = 7$; $P < 0.05$). I–V relationship for cells silenced for endogenous $Na_v1.5$, but untransfected, shown in red ($n = 5$). (*B*) Boltzmann fits of the voltage-dependent inactivation (dashed lines) of the p.H1849R channels (blue circles) demonstrated a depolarizing shift in the $V_{1/2}$ relative to WT (black circles; −101.2 vs. −107.9 mV; $P < 0.05$). Boltzmann fits of the voltage-dependent activation profile (solid blue and black lines) showed similar $V_{1/2}$ values (−61.9 vs. −62.4 mV for WT and p.H1849R, respectively; P = N.S.). R^2 values for all fits >0.99. (*C*) I_{Na} elicited from $Na_v1.5$ p.H1849R and FGF12 expressed in HEK293 cells (blue, $n = 14$) displayed significantly larger I_{Na} density relative to currents from cells expressing WT $Na_v1.5$ (black, $n = 11$; $P < 0.01$ for test voltages as indicated). (*D*) Boltzmann fits of the steady-state voltage-dependent inactivation of the p.H1849R channels expressed with FGF12 (blue triangles, $n = 13$) demonstrated a depolarizing shift in the $V_{1/2}$ relative to WT expressed with FGF12 (black circles, $n = 12$; −67.7 vs. −76.2 mV; $^*P < 0.01$). (*E*) Boltzmann fits of the voltage-dependent activation profile show similar $V_{1/2}$ values [−56.0 vs. −55.7 mV for WT ($n = 11$) and p.H1849R ($n = 14$), respectively; P = N.S.]. Vm in *D* and mV in *E*. (*F*) Scaled exemplar traces at a test potential of −40 mV and one-exponential fits of the decay phases for the p.H1849R channels expressed with FGF12 (blue solid line and dashed line, respectively) and for the WT $Na_v1.5$ channels expressed with (black solid line and dashed line, respectively). (*G*) Decay time, τ, of the fits as in *H* ($n = 28$ for p.H1849R and $n = 21$ for WT; $^*P < 0.01$).

CT demonstrates a critical association between FGF13 Arg57 with its interaction pocket anchored by residue H1849 on the $Na_v1.5$ CT (Fig. 2 *A* and *B*). We tested the impact of p.H1849R on $Na_v1.5$/FHF interactions. Coimmunoprecipitation experiments using detergent-soluble lysates from mouse heart confirmed interaction of $Na_v1.5$ with FGF13 (Fig. 2 *C* and *D*). Further, pull-down experiments from detergent-soluble lysates from mouse heart demonstrated direct association of GST–$Na_v1.5$ C terminus with FGF13 (Fig. 2*E*). The p.H1849R variant altered the interaction with FHFs. First, in the context of a GST–$Na_v1.5$ CT fusion protein, the variant reduced binding for FGF13 from detergent-soluble lysates from mouse heart (Fig. 2*E*). We observed no binding of GST alone for FGF13. Second, in vitro binding assays demonstrated similar results with all FHFs: radiolabeled FGF12, FGF13, and FGF14 all showed reduced interaction with GST–$Na_v1.5$ CT harboring the p.H1849R variant or GST alone (Fig. 2*F*). We quantified the impact of the $Na_v1.5$ p.H1849R variant on FHF binding using isothermal titration calorimetry. Purified WT $Na_v1.5$ C terminus and FGF13 associated with high affinity (K_d = 17 nM; Table S1; Fig. 2*G*). In contrast, purified $Na_v1.5$ C terminus p.H1849R showed ∼100-fold decrease in affinity for purified FGF13 (K_d = 1.9 μM; Table S1; Fig. 2 *H* and *I*). In summary, $Na_v1.5$ p.H1849R is associated with familial arrhythmias and significantly alters the interaction of $Na_v1.5$ with FHF family proteins.

Creation of in Vivo Model to Investigate Human *SCN5A* Variants. To analyze the consequences of altered interaction between the $Na_v1.5$ p.H1849R variant and FHFs on select I_{Na} properties, we created a new mouse model for inducible cardiomyocyte $Na_v1.5$ silencing. *Scn5a* knock-in mice were created with *loxP* sites flanking *Scn5a* exons 10 and 13 (Fig. S1) to silence *Scn5a* expression in the presence of *Cre* recombinase. Following birth, cardiomyocytes isolated from neonatal $Scn5a^{f/f}$ mice were cultured and analyzed for $Na_v1.5$-dependent current ± Adv-*Cre* transduction. Though we observed robust I_{Na} in mock-transduced myocytes, we detected only negligible I_{Na} in cultures transduced with Adv-*Cre* (Fig. S1). Consistent with these data, we observed diminished $Na_v1.5$ by immunoblot and immunostaining of Adv-*Cre*–infected myocytes (Fig. S1). Despite I_{Na} loss, myocytes remained healthy and viable. Notably, introduction of exogenous WT $Na_v1.5$ in these cultures resulted in rescue of I_{Na} to approximately physiological levels ($Scn5a^{f/f}$ myocyte $I_{Na,peak}$: 115.2 ± 8.4 pA/pF; $Scn5a^{f/f}$ + Adv-*Cre* + $Na_v1.5$ $I_{Na,peak}$: 142.2 ± 18.2 pA/pF). Thus, this new system is sufficient to analyze I_{Na} properties of putative $Na_v1.5$ variants.

$Na_v1.5$ p.H1849R Alters $Na_v1.5$ Steady-State Inactivation in Primary Myocytes. FHF proteins regulate Na_v channel steady-state inactivation (21, 29), and knockdown of FHFs in cardiomyocytes results in a hyperpolarizing shift in steady-state interaction (24, 27). We therefore used the $Scn5a^{f/f}$ myocyte system to test the human $Na_v1.5$ H1849R variant for alterations in I_{Na} inactivation properties. As noted above, I_{Na} levels in Adv-Cre–infected $Scn5a^{f/f}$ myocytes (GFP-positive) are rescued by expression of exogenous $Na_v1.5$ (Fig. 3*A*). Of note, expression of $Na_v1.5$ p.H1849R at equivalent levels resulted in an increased I_{Na} density relative to WT $Na_v1.5$ (Fig. 3*A*; increased ∼34%; $P < 0.05$). Further, we observed a significant ∼6.7-mV depolarizing shift in the $V_{1/2}$ of voltage-dependent inactivation of the p.H1849R variant compared with the WT channel (−101.2 vs. −107.9 mV; $P < 0.05$; Fig. 3*B*). Because FHF knockdown in cardiomyocytes leads to a hyperpolarizing shift in steady-state inactivation, these results suggested that the p.H1849R variant maintains an altered interaction with FHFs despite the reduced affinity noted in binding experiments with the isolated $Na_v1.5$ CT and FHFs (Fig. 2). We observed no difference in voltage dependence of activation of WT $Na_v1.5$ compared with $Na_v1.5$ p.H1849R (Fig. 3*B*), consistent with the lack of effect of FHFs on $Na_v1.5$

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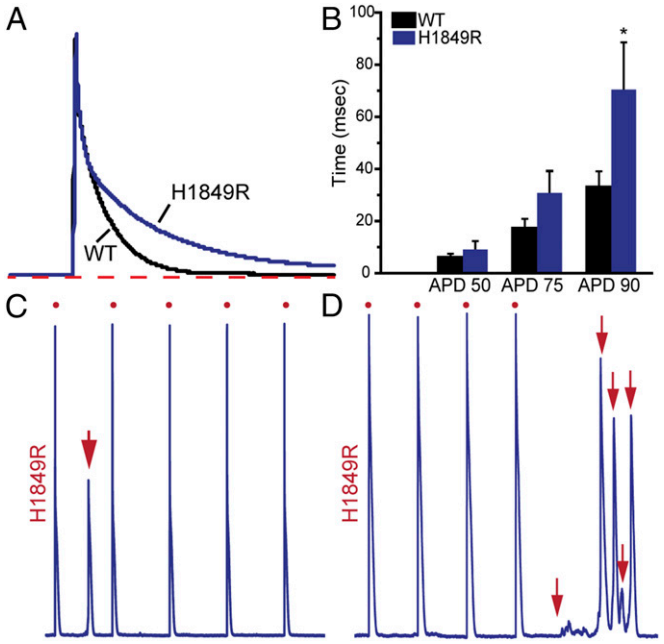

Fig. 4. $Na_v1.5$ p.H1849R prolongs AP duration and elicits spontaneous activity in murine myocytes. (*A* and *B*) Compared with myocytes expressing WT $Na_v1.5$, $Na_v1.5$ p.1849R-expressing myocytes display increased APD [APD_{90}; WT $Na_v1.5$: 33.1 ± 6.0 ms ($n = 7$ myocytes); $Na_v1.5$ p.H1849R: 70.4 ± 12.7 ms ($n = 7$ myocytes)]. (*C* and *D*) Unlike myocytes expressing WT $Na_v1.5$ ($n = 7$), $Na_v1.5$ H1849R-expressing myocytes displayed spontaneous activity during pacing (myocytes paced at 0.5 Hz in *C*) or spontaneous depolarizations following pacing [spontaneous activity observed following pause following 10 stimulations (red points) at 1.0 Hz in *D*].

interaction in cardiomyocytes (24). In summary, consistent with past work linking FHFs with regulation of Na_v channel steady-state inactivation (21, 24, 27, 29), $Na_v1.5$ p.H1849R displays aberrant steady-state inactivation in primary myocytes.

$Na_v1.5$ p.H1849R Displays Arrhythmogenic I_{Na} Properties. Patients harboring *SCN5A* variants with LQT3 display signature QT interval prolongation associated with altered $Na_v1.5$ regulation. To date, two molecular mechanisms underlying aberrant I_{Na} regulation in LQT3 are described. LQT3 is primarily caused by *SCN5A* variants that alter the fast inactivation gate of I_{Na}, resulting in channels that may reopen at later stages of the action potential (30). This depolarizing "late" I_{Na} current thus extends ventricular depolarization, often resulting in arrhythmogenic afterdepolarizations. Alternatively, human LQT3 may also be linked with *SCN5A* variants that slow $Na_v1.5$ channel inactivation (individual channels with longer open state duration) (31, 32), again extending ventricular depolarization and creating a substrate for arrhythmogenic afterdepolarizations.

To identify potential pathogenic mechanisms of the human $Na_v1.5$ H1849R variant in the complete absence of any potentially competing ionic currents, we analyzed I_{Na} signatures of WT $Na_v1.5$ and $Na_v1.5$ p.H1849R coexpressed with FGF12 in HEK293 cells (*SI Materials and Methods*). FGF12 was chosen as the FHF for this assay as it is the most abundant FHF in human heart (27). Consistent with findings in myocytes, we observed increased $I_{Na,peak}$ for $Na_v1.5$ p.H1849R compared with WT $Na_v1.5$ (Fig. 3*C*; $P < 0.05$). Further, we observed a depolarizing shift in steady-state inactivation for $Na_v1.5$ p.H1849R compared with WT $Na_v1.5$ (Fig. 3*D*; ~9-mV shift for $Na_v1.5$ p.H1849R relative to WT; −67.7 ± 1.9 vs. −76.8 ± 1.9 mV, respectively; $P < 0.05$). We did not observe differences in activation between $Na_v1.5$ and $Na_v1.5$ p.H1849R [Fig. 3*E*; WT: −57.1 ± 2.5 mV, p.1849R: −56.0 ± 1.5 mV; P = not significant (N.S.)]. Most notably, we observed a striking slowing of inactivation of $Na_v1.5$ p.H1849R compared with WT $Na_v1.5$ (Fig. 3 *F* and *G*; ~fivefold increase in decay time for $Na_v1.5$ p.H1849R compared with WT Nav1.5; $P < 0.05$). In summary, the $Na_v1.5$ p.H1849R variant has a number of gain-of-function phenotypes contributing to its arrhythmogenic properties, most notably a slowed rate of inactivation.

$Na_v1.5$ H1849R Alters Myocyte Membrane Excitability. Action potentials (APs) were measured in myocytes expressing WT $Na_v1.5$ or $Na_v1.5$ p.H1849R to define the relationship between the human variant and myocyte membrane excitability. In line with alterations in I_{Na} observed in both myocytes and heterologous cells, we observed a significant increase in AP duration (APD) at 90% repolarization (APD_{90}) for myocytes expressing $Na_v1.5$ p.H1849R compared with WT $Na_v1.5$-expressing myocytes (Fig. 4 *A* and *B*; $P < 0.05$). We observed no difference in resting membrane potential or peak transmembrane potential between the two groups (P = N.S.). Notably, consistent with the significant differences in APD_{90}, we observed spontaneous depolarizations (Fig. 4 *C* and *D*) in $Na_v1.5$ p.H1849R myocytes but not in myocytes expressing WT $Na_v1.5$ (WT $Na_v1.5$: 0% myocytes; $Na_v1.5$ p.H1849R: ~43% myocytes; $P < 0.05$). In summary, consistent with altered I_{Na} and the human LQT3 phenotype, $Na_v1.5$ p.H1849R results in APD prolongation and arrhythmogenic spontaneous membrane afterdepolarizations.

Discussion

Cardiac ion channel dysfunction is tightly linked with congenital human arrhythmias. Though mutations in ion channel pore and gating regions represent the vast majority of pathogenic variants, a second class of arrhythmia variants alter the regulation of ion channels by cytosolic signaling or scaffolding proteins. In fact, these findings have revealed new paradigms for rare forms of human arrhythmia based on defects in ion channel-associated proteins. By integrating human clinical and genetic data with biochemical, cell biological, and electrophysiology findings, we identify a new mechanism for human cardiac Na_v channelopathy based on loss of binding to the FHF family of ion channel regulatory proteins. Individuals harboring the $Na_v1.5$ p.H1849R variant display LQT3 and AF. Functionally, $Na_v1.5$ p.H1849R displays an altered interaction with FHFs, resulting in increased I_{Na} peak, increased $Na_v1.5$ channel availability and, most critically, slowed inactivation. Together, these parameters provide a rationale for the human cardiac phenotypes. In summary, these new findings directly validate the FHF family of signaling proteins as critical for human cardiac excitability. Further, these data illustrate the likely multifunctional roles of FHF proteins for the regulation of membrane excitability across diverse cell types.

The FHFs have gained significant attention due to their role in regulating Na_v and Ca_v channels (24, 29, 33–36), as well as their links with human excitable cell disease. In neurons, FHF dysfunction has been linked with aberrant I_{Na} phenotypes and human FGF14 variants cause spinocerebellar ataxia 27 (25). Further, the FGF13 locus has been linked with nonspecific forms of X-linked mental retardation (26). Finally, relevant for this study, a missense variant in FGF12 linked with aberrant $Na_v1.5$ function was recently associated with Brugada syndrome (27). However, to date, no human Na_v channel variants that alter association with FHF proteins have been linked with human disease. Of note, though FGF12–14 all directly associate with Na_v channels, each display unique Na_v channel regulatory properties that may differentially tune Na_v channel expression, trafficking, current density, availability, and/or persistent current ($I_{Na,L}$), depending on the cell type and Na_v channel isoform (29, 33, 34). Given these data, the differences in clinical phenotypes are not surprising, but raise important caveats regarding data interpretation.

The molecular mechanisms underlying human LQT3 may be multifactorial but are generally classified into two categories. The primary mechanism underlying LQT3 is altered $Na_v1.5$ fast

inactivation (30). As noted above, in this mechanism, mutant $Na_v1.5$ channels display an increased probability for reopening, resulting in inward depolarizing I_{Na} during the plateau phase of action potential ($I_{Na,late}$), lengthening of repolarization, and susceptibility to arrhythmogenic afterdepolarizations. However, though less common, slowed inactivation, resulting in $Na_v1.5$ channels with increased time in the open state, may also underlie LQT3 (31, 32). In our study, $Na_v1.5$ p.H1849R resulted in increased peak I_{Na} and increased I_{Na} availability (37, 38), consistent with known roles of FHFs in channel gating and/or internalization (21, 29). Most notably, the human $Na_v1.5$ p.H1849R variant displayed a striking signature of slow inactivation compared with WT $Na_v1.5$. Consistent with these data, compared with WT $Na_v1.5$, myocytes harboring the $Na_v1.5$ p.H1849R displayed increased APD and spontaneous afterdepolarizations even in the absence of adrenergic stimulation. Thus, our findings illustrate that $Na_v1.5$ p.H1849R is an I_{Na} gain-of-function mutation through the slowing of the rate of inactivation and increasing $Na_v1.5$ availability during the AP plateau phase, thus prolonging APD and producing proarrhythmic afterdepolarizations. These findings are consistent with prior reports linking I_{Na} slow inactivation and increased availability with both LQT3 (31, 32) and AF (38) as well as LQT associated with α1 syntrophin gene variants (37).

In summary, our data provide, to our knowledge, the first evidence for human disease based on Na_v channel gene variants that block FHF regulation. Our combined data support altered FHF binding as the mechanism for arrhythmia for $Na_v1.5$ p.H1849R. Further, recent unbiased structural data identify $Na_v1.5$ H1849 as a central residue for $Na_v1.5$/FHF association. Although the endogenous concentrations of FHFs and Na_v channel α-subunits in cardiomyocytes are not known, it is reasonable to assume that an ~100-fold decrease in affinity between the core domain of the FHF and the mutant $Na_v1.5$ C-terminal domain (CTD), the key interaction sites identified on each molecule (23), would affect FHF-dependent channel function. This hypothesis is consistent with the previous observation that an affinity-reducing mutation within the FHF core, at the Na_v channel interaction site, similarly confers altered Na_v channel function in neurons (36). However, we note that this variant may also alter $Na_v1.5$ folding and biophysical function independent of, or in conjunction with, FHF-mediated effects. Again, it will be critical in future in vivo experiments to dissect these specific points. In closing, our findings underscore the complexity and heterogeneity in the presentation of congenital arrhythmia, particularly as it relates to $Na_v1.5$ and its growing list of regulatory proteins.

Materials and Methods

Approval for use of human subjects was obtained from the Institutional Review Board of Ohio State University, and subjects provided informed consent. Genomic DNA was extracted from peripheral blood lymphocytes of the proband, proband's sister, mother, niece, and grandmother. A full LQTS genetic panel screened for mutations in 12 known disease-causing genes: *KCNQ1*, *KCNH2*, *SCN5A*, *ANK2*, *KCNE1*, *KCNE2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, and *SNTA1*. Sequencing identified a heterozygous A > G nucleotide transition in exon 28 in the *SCN5A* gene, producing a histidine-to-arginine amino acid change at the 1849 residue in the $Na_V1.5$ protein.

For animal experiments, animals were handled according to approved protocols and animal welfare regulations of the Institutional Animal Care and Use Committee of The Ohio State University. Detailed descriptions of all materials and methods are provided in *SI Materials and Methods*.

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