



Variant of SCN5A Sodium Channel Implicated in Risk of Cardiac Arrhythmia

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ErbB receptor function by binding receptor but failing to induce signaling.

The nature of the domain II/IV interaction, which is mediated entirely by a short hairpin loop from domain II, suggests another approach to modulating ErbB receptor behavior. Cyclic or linear peptides corresponding to this loop region (or the pocket on domain IV) or their analogs may be able to disrupt this interaction and potentiate ligand binding. Conversely, if ligand binding requires breaking of the domain II/IV interaction and the domain II loop then participates in interreceptor interactions, such peptides may antagonize signaling. Future experiments are needed to evaluate these strategies.

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26. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Materials and Methods
Figs. S1 and S2

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Variant of SCN5A Sodium Channel Implicated in Risk of Cardiac Arrhythmia

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Every year, ~450,000 individuals in the United States die suddenly of cardiac arrhythmia. We identified a variant of the cardiac sodium channel gene *SCN5A* that is associated with arrhythmia in African Americans ($P = 0.000028$) and linked with arrhythmia risk in an African-American family ($P = 0.005$). In transfected cells, the variant allele (Y1102) accelerated channel activation, increasing the likelihood of abnormal cardiac repolarization and arrhythmia. About 13.2% of African Americans carry the Y1102 allele. Because Y1102 has a subtle effect on risk, most carriers will never have an arrhythmia. However, Y1102 may be a useful molecular marker for the prediction of arrhythmia susceptibility in the context of additional acquired risk factors such as the use of certain medications.

Cardiac arrhythmias are a common cause of morbidity and mortality (1). Myocardial infarction, cardiac ischemia, cardiomyopathy, and many medications are common risk factors for life-threatening cardiac arrhythmias. However, not all individuals with a specific risk factor develop arrhythmias, and the reasons for this variability in response are not understood. One possibility is that genetic factors modulate arrhythmia risk in the setting of common, extrinsic factors (2).

The *SCN5A* gene encodes α subunits that form the sodium channel responsible for initiating the cardiac action potential (3). Mutations in *SCN5A* have been implicated in rare, familial

forms of cardiac arrhythmia, including long QT syndrome (4, 5), idiopathic ventricular fibrillation (6), and cardiac conduction disease (7–9). To identify common polymorphisms that increase the risk of arrhythmia in the general population, we screened DNA samples obtained from individuals with nonfamilial cardiac arrhythmias. In one case, a 36-year-old African-American woman (individual 5, table S1) with idiopathic dilated cardiomyopathy and hypokalemia developed prolongation of the corrected QT (QTc) interval and torsade de pointes ventricular tachycardia while on the anti-arrhythmic agent amiodarone (Fig. 1A). Prolongation of the QT interval is associated with an increased risk of life-threatening ventricular tachyarrhythmias (9). Single-strand conformation polymorphism (SSCP) and DNA sequence analyses (10) revealed a heterozygous transversion of C to A in codon 1102 of *SCN5A*, causing a substitution of serine (S1102) with tyrosine (Y1102). S1102 is a conserved residue located in the intracellular sequences that link domains II and III of the channel (fig. S1).

To determine the frequency of Y1102 in the general population, we used SSCP to screen DNA samples obtained from controls (10). Y1102 was observed in 19.2% of West Africans and Caribbeans (90/468). Eighty-

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revealed subtle differences in gating. We recorded a small (-4.5 mV), but significant, negative shift in the voltage dependence of activation in Y1102 [$V_{1/2}$ (voltage at which activation of the channel is half maximal) = -26.6 ± 1.3 mV, $n = 8$ (S1102); $V_{1/2} = -31.1 \pm 1.8$ mV, $n = 6$ (Y1102); $P = 0.05$] (Fig. 2B). Consistent with this shift of whole-cell current activation, we measured a significant increase in mean open probability at -40 mV for single Y1102 (0.42 ± 0.05 , $n = 4$) versus S1102 (0.30 ± 0.05 , $n = 4$) channels ($P = 0.04$). Y1102 peak current-voltage (I - V) relations reflected this change in activation gating (Fig. 2B). We compared the effect of Y1102 on peak transient current (I_{peak}) and its effect on sustained (bursting) current (I_{sus}) measured during prolonged depolarization. Y1102 I_{peak} was greater than that for S1102 [505.9 ± 39.1 pA/pF, $n = 33$ (Y1102) versus 442.4 ± 32.4 pA/pF, $n = 31$

(S1102); $P = 0.21$]. There was a greater difference in I_{sus} [0.51 ± 0.06 , $n = 30$ (Y1102) versus 0.37 ± 0.047 , $n = 30$ (S1102); $P = 0.06$]. These data indicate that Y1102 has greater peak amplitude and, more importantly, a larger I_{sus} than S1102.

To determine if these small changes in I_{peak} and I_{sus} current were consistent with the Y1102-induced shift in voltage dependence of activation and the clinical presentation, we performed simulation analyses (10, 13, 14). In the simulation, a 1.5-fold increase in the activation rate caused a -4.5 mV shift in voltage dependence of activation and altered the peak (I - V) relation as observed experimentally (Fig. 2C). Increased activation did not alter the voltage dependence of inactivation. Furthermore, our simulation predicted a slight Y1102-induced increase in I_{peak} and I_{sus} (Fig. 2D), similar to that observed with

the experiment data. Experimentally, Y1102 induced a mean increase of 14.5% in I_{peak} , as compared with an increase of 15.4% in the simulation. Y1102 increased the mean I_{sus} by 37% experimentally and by 32% in simulation. The larger increase in I_{sus} as compared with I_{peak} resulted from an increased probability of opening in the burst mode. Non-bursting channels inactivate rapidly after activation, whereas bursting channels deactivate to closed, but available, states in which the propensity to reopen is enhanced by

Fig. 2. SCN5A Y1102 increases the rate of cardiac sodium channel activation. (A) Whole-cell S1102 (left) and Y1102 (right) currents recorded during step depolarization (-70 to $+10$ mV, 10-mV increments) from a holding potential equal to -100 mV. (B) Experimentally determined activation curves (left), peak I - V relations (center) ($n = 8$ for S1102, $n = 6$ for Y1102), and steady-state inactivation (right) (500-ms conditioning pulses, 10-mV increments; $n = 14$ for S1102, $n = 15$ for Y1102) are shown. In each panel, closed symbols represent Y1102 and open symbols represent S1102 channels. (C) Computer simulation of whole-cell current properties. Y1102 channels are simulated by increasing the channel activation rate 1.5-fold. This change in rate causes (i) a negative shift in voltage dependence of activation (left), (ii) larger peak microscopic current in the I - V curve (center), and (iii) no change in the voltage dependence of availability (right). (D) The effect of Y1102 on I_{peak} (left) and I_{sus} (measured at 150 ms, right) current. Each panel compares the mean ratio of experimentally determined Y1102 or S1102 currents with predicted ratios from simulated currents. The experimental ratios were determined from mean data as follows: I_{peak} was 442 ± 32 pA/pF for S1102 ($n = 31$) and 506 ± 39 pA/pF for Y1102 ($n = 33$), and the ratio of means was 14.5%; I_{sus} was 0.37 ± 0.04 pA/pF for S1102 ($n = 30$) and 0.51 ± 0.06 pA/pF for Y1102 ($n = 30$), and the ratio of means was 37.8%. The increased rate of channel activation for Y1102 had a larger effect on I_{sus} amplitude than it had on I_{peak} , and simulation and experimentally determined values were nearly identical.

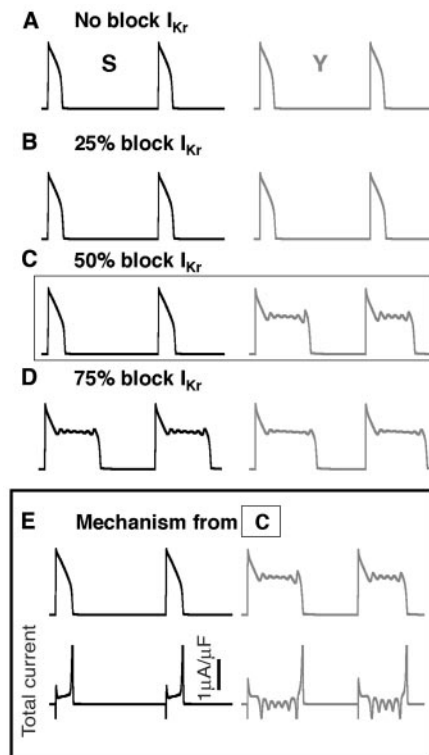
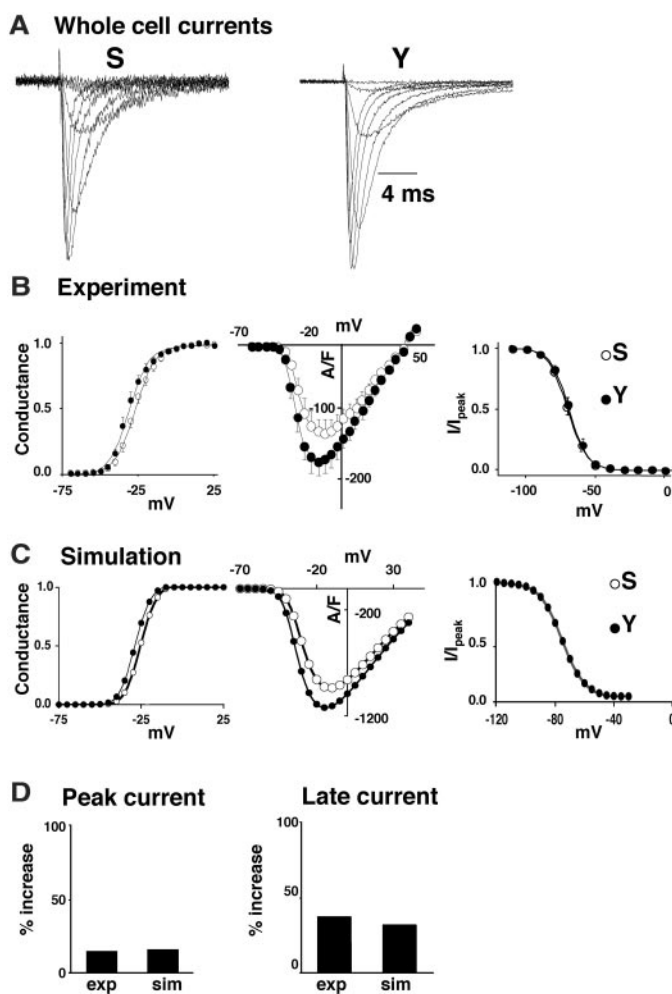


Fig. 3. SCN5A Y1102 increases arrhythmia susceptibility in the simulated presence of cardiac potassium channel blocking medications. Action potentials (19th and 20th after pacing from equilibrium conditions) for S1102 and Y1102 at cycle length = 2000 ms are shown for a range of I_{kr} block. I_{kr} is frequently blocked as an unintended side effect of many medications. Under the conditions of no block and a 25% I_{kr} block [(A) and (B), respectively], both S1102- and Y1102-containing cells exhibit normal phenotypes. As I_{kr} block is increased (50% block) (C), the Y1102 variant demonstrates abnormal repolarization. (D) With 75% I_{kr} block, both S1102 and Y1102 exhibit similar abnormal cellular phenotypes. The mechanism of this effect is illustrated in (E) by comparing action potentials in (C) with the underlying total cell current during the plateau phase of action potentials. Faster V_{max} (dv/dt) during the upstroke caused by Y1102 results in larger initial repolarizing current but not enough (due to drug block) to cause premature repolarization. This results in faster initial repolarization, which increases depolarizing current through sodium and L-type calcium channels. The net effect is prolongation of action potential duration, reactivation of calcium channels, EADs, and risk of arrhythmia.

Y1102. The probability of bursting was not affected by Y1102. Instead, Y1102 increased the probability that a channel will open in the burst mode by increasing the energetic favorability of the activation transition.

We next computed action potentials using simulated S1102 or Y1102 channels. The subtle changes in gating did not alter action potentials (Fig. 3). However, when we simulated a concentration-dependent block of the rapidly activating delayed rectifier potassium currents (I_{Kr}), a common side effect of many medications and hypokalemia, our computations predicted that Y1102 would induce action potential prolongation and early afterdepolarizations (EADs). EADs are a cellular trigger for ventricular tachycardia (15). Thus, computational analyses indicated that Y1102 increased the likelihood of QT prolongation, EADs, and arrhythmia in response to drugs (or drugs coupled with hypokalemia) (fig. S2) that inhibit cardiac repolarization.

We conclude that Y1102, a common SCN5A variant in Africans and African Americans, causes a small but inherent and chronic risk of acquired arrhythmia. The key to therapy is prevention. The identification of a common variant that causes a subtle increase in the risk of life-threatening arrhythmias will facilitate prevention through rapid identification of populations at risk. We estimate that 4.6 million African Americans carry Y1102 (16). Most of these individuals will never have an arrhythmia because the effect of Y1102 is subtle. However, in the setting of additional acquired risk factors, particularly common factors such as medications, hypokalemia, or structural heart disease, these individuals are at increased risk. Successful strategies for prevention, including avoidance of certain medications (17–19), maintenance of a normal serum potassium concentration (20), and beta-blocker therapy (21), are available. Additional, longitudinal studies will be required to confirm the predictive utility of Y1102.

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Supporting Online Material

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Mechanisms of Adaptation in a Predator-Prey Arms Race: TTX-Resistant Sodium Channels

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Populations of the garter snake *Thamnophis sirtalis* have evolved geographically variable resistance to tetrodotoxin (TTX) in a coevolutionary arms race with their toxic prey, newts of the genus *Taricha*. Here, we identify a physiological mechanism, the expression of TTX-resistant sodium channels in skeletal muscle, responsible for adaptive diversification in whole-animal resistance. Both individual and population differences in the ability of skeletal muscle fibers to function in the presence of TTX correlate closely with whole-animal measures of TTX resistance. Demonstration of individual variation in an essential physiological function responsible for the adaptive differences among populations is a step toward linking the selective consequences of coevolutionary interactions to geographic and phylogenetic patterns of diversity.

Complex phenotypes such as performance or resistance typically comprise physiological, morphological, and behavioral components (1). Although selection acts directly on complex phenotypes themselves (2, 3), it is ultimately the evolution of these underlying components that shapes patterns of adaptation among taxa. The indirect evolutionary response of physiological mechanisms and other causal factors requires individual variation in physiology that is correlated with a performance measure that is in turn correlated with individual differences in fitness. This cascade of effects links physiology to fitness and, given appropriate genetic variation in physiology, is thought to lead to adaptive diversification in performance and the underlying factors that influence it (3). Considerable effort in integrative biology has been devoted to understanding the mechanistic basis of complex traits that differ among populations or species (4). However, few studies have successfully linked presumably adaptive differences in physiological function among

populations to the analogous individual and genetic variation within populations that is required for natural selection to explain adaptive diversification in physiological traits.

Coevolutionary interactions generate particularly rapid and complex patterns of adaptive diversification among populations (5); theoretical developments emphasize the critical role that geographic variation in selection plays in determining the dynamic of coevolution and predict that coevolutionary outcomes will vary markedly among populations of the same species (5–7). Coevolutionary interactions therefore are particularly suitable for studying the link between mechanisms and patterns of adaptation. We investigated a performance trait, tetrodotoxin (TTX) resistance, at the phenotypic interface of the “arms race” between a garter snake predator, *Thamnophis sirtalis*, and its toxic prey, newts of the genus *Taricha*.

The skin of *Taricha granulosa* contains TTX, a potent neurotoxin that blocks voltage-gated sodium channels in nerve and muscle tissue, thereby inhibiting the propagation of action potentials (APs) and paralyzing nerve and muscle function (8, 9). TTX binds to sodium channels in many different tissues, but death from TTX intoxication usually results from respiratory failure (10, 11). Some

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