Title page

Ethnic-related SCN5A polymorphisms shape the *in vitro* pharmacological action of amiodarone upon Nav1.5

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Amiodarone and ethnic-related SCN5A polymorphisms

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Abbreviations:
action potential (AP)
amiodarone (AMIO)
atrioventricular (AV)
current-voltage relationship (IV)
Dimethyl sulfoxide (DMSO)
maximum conductance (G_{MAX})
maximum current (I_{MAX})
wild-type (WT)
Abstract

Nav1.5-derived Na\(^+\) current (I\(_{Na}\)) exerts a pivotal role in the depolarization phase of cardiomyocytes’ action potential, and therefore, changes in I\(_{Na}\) can contribute to fatal arrhythmias. Nav1.5 displays naturally occurring ethnic-related polymorphisms, which might alter the functioning and pharmacology of the channel. Some studies have shown how single nucleotide polymorphism can change the response to antiarrhythmic drugs. Investigations on the role of Nav1.5 in arrhythmogenesis, associated with its functional polymorphisms, are currently growing, as well as the possible variability in the antiarrhythmic pharmacotherapy among ethnic-groups. The influence of the ethnic-related polymorphisms (S524Y, S1103Y, R1193Q, V1951L) on the responsiveness, selectivity, and pharmacological efficacy of the clinically used antiarrhythmic, amiodarone (AMIO), is not completely known. Our objectives were to analyze biophysical and pharmacological aspects of four ethnic-related polymorphisms before and after exposure to AMIO. Polymorphisms caused reduced AMIO potency compared to wild-type (WT) that can vary up to 4x between them. AMIO shifted the voltage dependency for current inactivation, without significant effect in voltage-dependent activation to a similar extend in WT and polymorphisms. The recovery from inactivation was altered between the polymorphisms when compared to WT. Finally, the use-dependency of AMIO differed between studied groups, especially at a more depolarized cell membrane. Thus, our work may guide future studies focusing on the efficiency of AMIO in treating different arrhythmias and to establish more individualized guidelines for its use depending on the Nav1.5-polymorphism, after validating our findings using in vivo studies.
Significance Statement

SCN5A gene encodes the alpha subunit of Nav1.5, the main cardiac voltage-gated Na+ channel. Interestingly, ethnic-related polymorphisms are found in SCN5A. Amiodarone is used in clinical practice and some of its effects are attributed to interaction with Nav1.5. Important, amiodarone efficacy is variable among patients. Here we show that Ethnic-related SCN5A polymorphisms lead to altered Nav1.5-amiodarone interaction, which may be the cause for the variable efficacy observed in clinical usage of amiodarone.
1 Introduction

SCN5A is the gene responsible for encoding the pore-forming alpha subunit of the human cardiac voltage-gated Na\(^+\) channel, Nav1.5 (Zimmer et al., 2014). Changes in Nav1.5 sequence can lead to modification of its biophysical properties (Tan et al., 2005), (Hu et al., 2015). Nav1.5-derived Na\(^+\) current (I\(_{\text{Na}}\)) exerts a pivotal role in the depolarization phase of cardiomyocytes action potential (AP), and therefore, modifications in the I\(_{\text{Na}}\) can cause electrical disturbances of myocytes, and contribute to the occurrence of cardiac arrhythmias (Veerman et al., 2015), (Veltmann et al., 2016). Indeed, cardiac arrhythmias, such as the Long QT Syndrome Type 3, atrial fibrillation, and Brugada Syndrome have been associated with abnormal biophysical properties of Nav1.5 (Veerman et al., 2015), (Veltmann et al., 2016), (Kruger and Isom, 2016).

An interesting aspect of Nav1.5 is the existence of naturally occurring polymorphisms that might alter the functioning and pharmacology of the channel (Tan et al., 2005), (Makielski et al., 2003). Single nucleotide polymorphism is the most frequent genetic alteration, and mutation of a single nucleotide may or may not trigger the formation of a new product with loss or gain of Nav1.5 function. Intriguing, some of these polymorphisms can be ethnic-related such as S524Y, S1103Y, R1193Q, and V1951L (Tan et al., 2005), (Ackerman et al., 2004), (Tester and Ackerman, 2009).

The ethnic-related polymorphisms, S524Y and S1103Y, have a prevalence of 6% and 13%, respectively, in the Afro-descendant population (Tan et al., 2005), (Splawski et al., 2002). Considering the V1951L and R1193Q polymorphisms, they are prevalent (7% and 0.3%, respectively) in whites and Hispanic people. Moreover, the V1951L polymorphism has a prevalence of 16% in the Asian population.
(Ackerman et al., 2004). Each one of these polymorphisms might lead to specific phenotypes with a singular consequence for heart function and therapy. For example, some studies have shown how a single nucleotide polymorphism can change the response to antiarrhythmic drugs commonly used in clinical practice (Shinlapawittayatorn et al., 2011), (Sardar et al., 2014), (Sardar et al., 2016).

The interest in biomedical investigations on the role of Nav1.5 in arrhythmogenesis, associated with its functional polymorphisms, is currently growing, as well as the possible variability in the antiarrhythmic pharmacotherapy among ethnic groups (Sardar et al., 2014), (Sardar et al., 2016). To date, the influence of the ethnic-related polymorphisms on the responsiveness, selectivity, and pharmacological efficacy of antiarrhythmic drugs targeting Nav1.5 is not completely known. The antiarrhythmic drug amiodarone (AMIO), for example, is a classic antiarrhythmic agent belonging to class III and has diverse pharmacological action mechanisms because it also inhibits peak Nav1.5 ($I_{Na-p}$) (class I activity), $I_k$ (class III activity), $I_{Ca}$ (class IV activity), and α-β adrenoceptors (class II activity), being used in patients with ventricular tachyarrhythmias, heart failure, and recurrent fibrillation (Sardar et al., 2014), (Sardar et al., 2016), (Lafuente-Lafuente et al., 2015). Clinically, AMIO represents about 45% of all prescriptions in the United States (Zimetbaum, 2012). Prospective clinical trials demonstrated that among all antiarrhythmic drugs currently used in the management of fibrillation, AMIO has the greatest (~65%) potential for maintaining sinus rhythm, compared with other antiarrhythmics, such as sotalol and propafenone (Vamos and Hohnloser, 2016).

Considering the AMIO’s clinically intense use and the ethnic-related polymorphisms, our objectives in this study were to analyze biophysical and pharmacological aspects of S524Y, S1103Y, R1193Q, and V1951L before and after
exposure to AMIO. We understand that our data can contribute to advances the knowledge about how ethnic-related polymorphisms affect the interaction of AMIO with Nav1.5, which can provide evidence to design a more accurate and individualized pharmacological strategy to treat arrhythmic syndromes.
2 Materials and Methods

2.1 Ethics

All experimental protocols were approved by our institutional committee that regulates the use of laboratory procedures (CEP # 1869170619).

2.2 Cell culture, polymorphisms and transfection

For cellular procedures, it was used the Human Embryonic Kidney cells (HEK293) that lack functional macroscopic $I_{Na}$. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Burlington, ON) with 4.5 g/L of D-(+)-Glucose and 10% fetal bovine serum, and 1% penicillin/streptomycin supplementation. The cells were maintained at 37 °C and 5% CO$_2$ atmosphere in an incubator. Human wild-type Nav1.5 (WT), and all polymorphisms studied S524Y, S1103Y, R1193Q, and V1951L were cloned into the BamH1 and HindIII restriction sites of the pcDNA 3.1 vector (Invitrogen), purchased from GenScript Limited (Hong Kong). In this study, all Nav1.5 variants were evaluated using the backbone of SNC5A- isoform NCBI reference number XM_006713282.2 (containing 2016 residues including Q1077).

For transfection procedure, HEK293 cells were kept in 60 mm culture at 60-80% confluence, maintained for 5 h in 800 μL Opti-MEM (Thermo Fisher Scientific) added with 4 μL Lipofectamine 2000, 1 μl PLUS™ Reagent (Invitrogen), and 1 μg of each plasmid cDNA. After 5 h period, the Opti-MEM was replaced by DMEM culture medium. Patch-clamp recording was performed after 48 h.

2.3 Whole-cell patch-clamp recording

All procedures were performed at room temperature (~22-25 °C). The EPC-10.2 patch-clamp amplifier (HEKA Elektronik, Germany), and Axopatch 200B amplifier (Molecular Devices), in the voltage-clamp mode were used to record the
macroscopic $I_{\text{Na}}$ via the PACTHMASTER acquisition software (HEKA Elektronik, Germany) or Clampex 10.7 (Molecular Devices, United States of America). During experiments, transfected cells were replated onto glass coverslips and mounted in a recording chamber filled with extracellular solution containing (in mM): 130 NaCl; 1.8 CaCl$_2$; 5 CsCl; 1.2 MgCl$_2$; 10 HEPES; 5 Glucose, pH = 7.4. Patch pipettes tip resistance was set to 2.5-3.0 MΩ, and series resistance was typically 3-5 MΩ. Cells with series resistance > 7 MΩ were not considered in order to prevent voltage-clamp errors. Electronic compensation of series resistance (60-70%) was applied given the fast kinetics and the steep voltage dependence of $I_{\text{Na}}$. In addition, patch pipettes were filled with an intracellular solution, containing (in mM): 50 aspartic acid; 60 CsCl; 5 Na$_2$ATP; 11 EGTA; 10 HEPES; 4.27 CaCl$_2$; 1 MgCl$_2$; pH = 7.2. After the whole-cell configuration stabilization, cells were kept for 2 min at rest to allow proper intracellular solution dialysis. Records were low-pass filtered (cut-off frequency 2 kHz) and digitalized at 10-20 kHz. HEK293 cells typically fall under 10-30 pF cell capacitance range.

The pharmacological potency of AMIO was evaluated for WT and Nav1.5 ethnic-polymorphisms S524Y, S1103Y, R1193Q, and V1951L. It was applied crescent concentrations of the drug (in µM) 1; 10; 30; 100; 1000. A square pulse to -20 mV from a holding potential of -120 mV, and 1 Hz stimulation frequency was used to access the tonic effect of AMIO, which is commonly achieved after 2 min stimulation. Peak inward current level prior to AMIO challenge was considered 100%, which was our control for the dose-response curve analysis. Percentage of drug blockage was determinate as the fraction of reduced peak inward current after steady-state level following AMIO exposition was achieved, normalized by control peak inward current. After normalization, the equation $y = A1 + (A2-A1)/(1 +$
$10^{((\log x_0-x)*p)}$ was fitted for WT and each sodium channel polymorphism curves, in which $A_1$ (bottom asymptote) and $A_2$ (top asymptote) were fixed at 0 and 1, respectively. Data were analyzed using OriginPro 2018. Voltage dependency for current activation was accessed by 25 ms square pulses ranging from -80 to +37 mV, with 3 mV increment and at 1 Hz frequency and from a holding potential of -120 mV. Boltzmann equation $I = \left( E_m - E_{rev} \right) \frac{G_{max}}{1 + e^{\left( \frac{V_{50} - E_m}{k} \right)}}$ was fitted to the current-voltage relationship (IV) where $E_{rev}$ is the predicted reversal potential of the current, ($V_{50}$) is the voltage in which half of the current is activated, $G_{MAX}$ is the estimated maximum conductance and $k$ is the slope of the curve. Conductance at each tested potential ($G$) was normalized by ($G_{max}$). $G/G_{max}$ plots were then fitted using a Boltzmann equation: $G/G_{max} = \frac{1}{1 + e^\left( \frac{V_m - V_a}{k_a} \right)}$. $E_m$ is the membrane potential, $V_a$ is the voltage in which half of the maximum conductance is achieved and $k_a$ represents the slope of the curve. Data were analyzed using OriginPro 2018.

Time to peak of $I_{Na}$ was computed as the time of pulse beginning until the maximum inward current was achieved in each tested potential. Time to $I_{Na}$ decay was calculated as the time to peak $I_{Na}$ to decay 50% of its value for each tested membrane potential.

Voltage-dependent steady-state inactivation was studied by a 50 ms duration and -20 mV amplitude test pulse immediately after a long pre-pulse (2000 ms), ranged from -120 mV to 20 mV, with 5 mV increment at each step with 1 s interval between sweeps, in order to achieve a stationary voltage-dependent inactivation. Peak currents obtained from the -20 mV test pulse after each pre-pulse was normalized by maximum current ($I/I_{max}$) and fitted using a Boltzmann equation.
In this equation, $E_m$ represents the membrane voltages from the conditioning pulses, $V_h$ if the potential corresponding to half the maximum inactivation ($V_{ih}$) and slope factor ($k_h$). Data were analyzed using OriginPro 2018.

Window current was calculated from the area under the intersection of fitted voltage-dependent activation and steady-state inactivation curves normalized by peak $I_{Na}$. Boltzmann equation was fit to the curves.

Recover from inactivation was recorded as double-squared pulses (50 ms and 100 ms) to -20 mV from a holding potential of -120 mV with a variable inter-interval pulse: 1-10 ms, with 1 ms increment; 12-98 ms with 2 ms increment; 800-1000 ms with 100 ms increment. A two-exponential equation was fitted to the recovery from inactivation curves, as following: $Y=A_1*(1-exp(X/\tau_1)) + A_2*(1-exp(X/\tau_2))$ from which fast and slow time constants ($\tau_1$ and $\tau_2$) current recovery was obtained. $A_1$ and $A_2$ represent the initial quantities, the maximum fractional current recover for the fast and slow components, respectively. Data were analyzed using OriginPro 2018.

The estimated time constants were compared between groups before and after exposure to AMIO. In addition, the normalized fractional contribution of each component ($A_1$ and $A_2$) obtained from the exponential equations were compared between the experimental groups. Data were analyzed using OriginPro 2018.

The use-dependent block of $I_{Na}$ was evaluated by varying the stimulation frequency (1 Hz, 2 Hz, and 5 Hz) of square pulses (-20 mV, 100 ms). This protocol was run from a holding potential of -120 mV and -80 mV. In all protocols described above, Nav1.5-WT, and polymorphisms, were recorded before and after reach a stable tonic effect of AMIO (typically after 2-3 min) that was accompanied with square pulses to -20 mV from a holding potential of -120 mV. In all experiments, AMIO was solubilized from a stock of 20 mM solution in Dimethyl sulfoxide (DMSO).
2.4 Statistical analysis

OriginPro 2018 was used for all statistical analyses. Normally distributed data (using the Kolmogorov–Smirnov test) were analyzed using one-way, two-way ANOVA followed by Tukey’s post hoc test or Student T-test, as indicated in the figure legend or table. Proportion was evaluated using Chi-Square Test. A significance level of 5% was set in all analyses (p < 0.05). Data are expressed as mean ± standard deviation.
3 Results

3.1 Comparing the pharmacological potency of AMIO in Nav1.5 ethnic-related polymorphisms

As seen in Figure 1A, the sequence of Nav1.5 ethnic-related polymorphisms was aligned with other isoforms of Nav in the region of interest in which each polymorphism occurs. The schematic representation of Nav1.5 channels is marked with dashed squares that highlight the region from each variant is located and was constructed according to Pan et al. 2018 (Pan et al., 2018). The S524 is located in the DI-DII linker. The S1103 residue is positioned in the DII-DIII linker. The R1193 residue is located in the S1 transmembrane segment of the DIII domain, and finally, the V1951 residue is placed in the C-terminal domain of the Nav1.5 channel. Each one of the Nav1.5 polymorphisms was individually transfected to HEK293 cells, and the whole-cell patch-clamp technique was performed to record $I_{Na}$, in the presence or absence of AMIO. Figure 1B displays the percentage of transfected cells in which macroscopic $I_{Na}$ was observed, and no difference between all studied groups was observed, as expected since these Nav1.5 variants per se are not pathological ($p > 0.05$).

Then, the pharmacological potency of AMIO was evaluated for wild-type (WT) and Nav1.5 ethnic-related polymorphisms. Figure 2A shows the time course of AMIO effects on peak $I_{Na}$ elicited from a square pulse to $-20$ mV, from a holding potential of $-120$ mV. Transfected HEK293 cells were exposed to AMIO, and the representative traces of the time-course effect of AMIO on $I_{Na}$ are presented in Figure 2B. The summarized effects of AMIO percentage block of $I_{Na}$ are plotted in Figure 2C, and fitted with a symmetrical sigmoidal curve. The pharmacological potency of AMIO was estimated as the concentration required to reach 50% of the current block ($IC_{50}$). We
can observe that the highest pharmacological potency was detected in the control group (WT- $I_{Na}; IC_{50} = 31.7 \pm 11.3 \mu M$, 95% confidence interval, 9.5 to 53.9 uM). On the other hand, the potency of AMIO was lower in all polymorphisms compared to WT, the lowest being found in S524Y ($IC_{50} = 109.8 \pm 45.9 \mu M$, 95% confidence interval, 19.8 to 199.7 uM, $p < 0.05$ compared to control using two-way ANOVA) which displayed almost 4x less sensitivity to AMIO. R1193Q showed the highest potency among the studied polymorphisms ($IC_{50} = 77.6 \pm 28.4 \mu M$, 95% of confidence interval, 21.9 to 133.3 uM, $p < 0.05$ compared to control using two-way ANOVA), while S1103Y and V1951L had intermediate values ($IC_{50} = 98.6 \pm 29.8 \mu M$, 95% confidence interval, 40.2 to 157.1 uM, $p < 0.05$ compared to control using two-way ANOVA, and $IC_{50} = 93.1 \pm 21.9 \mu M$, 95% of confidence interval, 50.1 to 136.1 uM, $p < 0.05$ compared to control using two-way ANOVA, respectively). Hence, all ethnic-related polymorphisms showed a significant reduction in AMIO sensitivity compared to WT ($p < 0.05$). With these results, we conclude that AMIO displays different pharmacological sensitivity among the ethnic-related polymorphisms studied.

3.2 Effects of AMIO in the voltage dependency for current activation of Nav1.5 ethnic-related polymorphisms

A series of square pulses ranging from -80 to +37 mV (3 mV increments on each step at 1 Hz frequency) from -120 mV holding potential, was applied to generate a current-voltage (IV) relationship. Representative current traces of $I_{Na}$ activation followed by depolarizing steps are displayed in Figure 3A. The normalized IV plots can be seen in Figure 3B, the middle column. From the IV plots, the maximum conductance ($G_{MAX}$) of each cell was estimated using a Boltzmann equation fitted to the data (see methods). The calculated conductance at each
voltage step (G) was normalized by the maximum conductance, and a Boltzmann equation fitted to the G/G\textsubscript{MAX} curves (Figure 3C). G/G\textsubscript{MAX} is the distribution of the fractional conductance among the tested voltages, which represents the voltage dependency for channel activation. We observed that the voltage in which half of G\textsubscript{MAX} is achieved (V\textsubscript{a}) were similar in WT and all polymorphisms, although the slope factor was smaller in S524Y and larger in S1103Y when compared to WT (Table 1). However, challenging groups with 10 \(\mu\)M of AMIO altered neither the slope factor nor V\textsubscript{a}. Using the same patch-clamp recordings from Figure 3, we evaluated the impact of AMIO on the time to peak current (Supplementary Figure 1A) and on the time to 50% of current inactivation (Supplementary Figure 1B). AMIO was not able to change neither time to current peak nor time to 50% of current inactivation in WT and in the studied polymorphisms. Although it is interesting to note that prior to AMIO challenge we found a slower time for I\textsubscript{Na} peak and decay in S1103Y and R1193Q when compared to WT (Supplementary Figure 2A and 2B) and faster I\textsubscript{Na} decay for S524Y when compared to WT (Supplementary Figure 2A and 2B). Thus, these data suggest that AMIO has no significant impact on the transition from the closed to open state of the cardiac sodium channel in WT and in the studied polymorphisms.

3.3 Effects of AMIO in the stationary voltage-dependent inactivation of I\textsubscript{Na} of Nav1.5 ethnic-related polymorphisms

A series of preconditioning pulses ranging from -120 to 0 mV (6 mV increment on each step, at 1 Hz) from a -120 mV holding potential, and with 2000 ms, was applied to establish a stationary voltage-dependent inactivation of I\textsubscript{Na} (the remaining non-inactivated current was measured from a second test pulse, -20 mV). Figure 4 displays the representative traces from the test pulse, before (Figure 4A, left column) and after (Figure 4B, middle column) exposure to 10 \(\mu\)M AMIO. The orange thick
trace is the remaining current at -80 mV, highlighting the effects of AMIO in the stationary voltage-dependent inactivation of $I_{Na}$, in WT and tested polymorphisms. The $I_{Na}$ obtained after each preconditioning voltage ($I$) was normalized by the maximum current when the conditioning pulse was at -120 mV ($I_{MAX}$) and plotted as a function of the tested conditioning voltages ($I/I_{MAX}$) in Figure 4C, right column. $I/I_{MAX}$ is the distribution of the fractional current among the tested voltages, which represents the voltage dependency for channel inactivation. This stationary voltage-dependent inactivation can be compared from the voltage in which half of $I_{MAX}$ is achieved ($V_{h}$). A Boltzmann-like equation was fitted to the data (see methods section).

No difference was observed in $V_{h}$ from the Nav1.5 polymorphisms compared to WT before exposure to AMIO ($p > 0.05$). Moreover, in all variants, after AMIO exposure there was a negative shift in the $V_{h}$ of $I_{Na}$ similar to which recorded from the WT and polymorphisms. Interesting, slope factor is higher in WT (from $5.1 \pm 0.7$ to $6.4 \pm 0.5$, $p < 0.05$) and S1103Y (from $5.2 \pm 0.6$ to $7.1 \pm 1.3$, $p < 0.05$) after challenging with AMIO. Additional fitting parameters from all groups are listed in Table 2. From these results, we conclude that the AMIO negatively-shift induced on the WT isoform of Nav1.5 channel voltage-dependent inactivation is maintained among the Nav1.5 polymorphisms, after exposure to AMIO.

Since AMIO caused a negative shift on the inactivation curve of $I_{Na}$ we decided to calculate the window $I_{Na}$ prior and after AMIO exposure. Window current is considered as the area under the curve of intersection between voltage-dependent activation and steady-state inactivation curves. Boltzmann equation was fitted to both curves. Prior to fitting, data were normalized by maximum $I_{Na}$, using experimental data from Figures 3 and 4. As shown in Supplementary Figure 3, WT
and polymorphisms had a small, but detectable window $I_{\text{Na}}$. AMIO did not change the magnitude of the window $I_{\text{Na}}$ in none of the tested groups.

3.4 Effects of AMIO in the recover from inactivation of $I_{\text{Na}}$ of Nav1.5 ethnic-related polymorphisms

The fast and slow recovery of $I_{\text{Na}}$ from inactivation was accessed through a series of twin pulses (to -20 mV, from a holding potential of -120 mV), varying the interval between the test and the conditioning pulse (1-1000 ms). Since the conditioning pulse brings the majority of sodium channels to the inactivation state after being activated, we evaluated the fractional current that is recovered as a function of the time interval between the twin pulses. Figures 5A and 5B display the representative traces of $I_{\text{Na}}$ recovery from inactivation before (5A, left column) and after (5B, middle column) exposure to 10 µM AMIO. Only the first 10 sweeps, in which interval time was between 1-10 ms, were displayed to highlight the effects of AMIO in the fast-component ($\tau_1$) of inactivation recovery. The summarized data regarding the fast and slow recover from inactivation is presented in Figure 5C, right column. A two-component exponential equation was used to estimate the fast ($\tau_1$) (Table 3) and slow ($\tau_2$) (Table 4) time constants of the current recovery. In addition, the relative contribution ($A_1$ and $A_2$, see Table 5 and Table 6) of each recovery component to the peak current was studied.

S1103Y and R1193Q variants displayed slower $\tau_1$ compared to WT ($p < 0.05$, Table 3), while comparable $\tau_2$ was found in all groups prior to AMIO challenge. Moreover, AMIO slowed both the $\tau_1$ and $\tau_2$ in WT and all sodium channel variants, and S1103Y and R1193Q displayed slower kinetics of $\tau_1$ compared to WT. Also, AMIO exerted a significant effect on the relative contribution of $\tau_1$ and $\tau_2$ to the total current recovery, especially for S524Y, S1103Y, and R1193Q, in which the relative
contribution of $\tau_1$ was larger when compared to WT. From these data, we conclude that AMIO strongly slows the recovery from inactivation kinetics of all variants. In addition, AMIO changes the contribution of $\tau_1$ and $\tau_2$ to inactivation in an ethnic-related fashion.

3.5 Use-dependent block of $I_{Na}$ by AMIO among the Nav1.5 ethnic-related polymorphisms

The use-dependent block of $I_{Na}$ after exposure to 10 $\mu$M of AMIO was evaluated by square pulses (-20 mV), varying the stimulation frequency from 1 Hz, 2 Hz, and 5 Hz, from a holding potential of -120 mV (Figure 6). Figure 6A shows the representative traces of $I_{Na}$ recorded after exposure to AMIO. Current traces were normalized by the maximum current (at 1 Hz), for each group. Normalized currents were plotted as a function of the log of stimulation frequency (Figure 6B), and a linear regression was fitted to the data, from which the slope of the curve was used to compare the use-dependent block of AMIO among them. As shown in Figure 6B and Table 7, WT and all polymorphisms were well fitted after linear regression, with strong correlation, with $R^2$ typically > 0.85, as well as with Pearson’s correlation (typically below -0.9), indicating that there is a strong inverse correlation between increased frequency and the current block and they are comparable between all groups.

Finally, the use-dependent block of $I_{Na}$ by AMIO was further evaluated using a more depolarized holding potential of -80 mV, (which can mimic the membrane potential of atrial cardiomyocytes). Normalized current traces at the recorded frequencies are shown in Figure 7A, for WT and Nav1.5 polymorphisms. Figure 7B displays the summarized plots of the normalized currents recorded at -80 mV holding potential as a function of the stimulation frequency. As it can be seen in Table 8,
R1193Q showed no significant use dependency when membrane holding potential was -80 mV, while the other sodium channel variants showed a similar profile to WT. Combined, these data indicate $I_{Na}$ blockage by AMIO is used-dependent, and overall studied polymorphisms displayed a similar behavior as observed for WT, except for R1193Q variant.
4 Discussion

It is known that AMIO is a classical type III antiarrhythmic drug (Vaughan Williams, 1984), which was initially characterized as a blocker of rectifying K\(^+\) currents, and therefore, prolonging the AP duration (Vaughan Williams, 1975) and the QT interval (Torres et al., 1986). The blockage of Na\(^+\) channels can also decrease the probability of ectopic activity through the reduction of cardiomyocytes’ excitability (Heijman et al., 2017), (Heger et al., 1981), (Hamer et al., 1983). Then, the class I action of AMIO can also substantially contribute to its antiarrhythmic efficacy. Today, it is known that AMIO has a broader action on ion channels found in myocytes displaying antiarrhythmic properties of class I, II, IV (Heijman et al., 2017).

Here, we accessed the pharmacological potency of AMIO in blocking I\(_{\text{Na}}\) among naturally occurring Nav1.5 ethnic-related polymorphisms. AMIO potency can be reduced up to 4x in variants when compared to WT. Taking as an example the African-selective polymorphisms, S1103Y and S524Y, both displayed 3-4x less sensitivity to AMIO when compared to WT. This feature can become clinically relevant if such a difference in the pharmacological potency prevents or at least, significantly attenuates the AMIO-class I activity, which may lower the efficacy of AMIO in treating ventricular or supraventricular arrhythmias in individuals harboring these polymorphisms. This also can be particularly important when considering the lower bioavailability and dosing of long-term oral treatments with AMIO (Hamilton et al., 2020). Following this rationale, some long-term AMIO antiarrhythmic effects were partially associated with the prevention of triggered mechanisms (Singh et al., 1984), and the blockage of I\(_{\text{Na}}\) is known to create stabilization of the membrane potential, driven by the reduction of overall cell excitability (Kahlig et al., 2014), (Shaw and Rudy, 1997a), (Shaw and Rudy, 1997b).
Next, we compared the electrophysiological phenotype of four common ethnic-related Nav1.5 polymorphisms and the effects of AMIO. Some biophysical aspects of the Nav1.5 polymorphisms analyzed here have been investigated in other studies. Using Q1077 splice variants, previous studies reported no change in the $V_a$ of V1951L (Tan et al., 2005), which is consistent with our data. The same work reported no change in the $V_a$ of S524Y, a positive shift of S1103Y, and a negative shift of R1193Q. However, in our study, $V_a$ from all variants was comparable to WT. Our data are consistent with other works both for the African-related S1103Y (Plant et al., 2006), as for the R1193Q (Abe et al., 2018). However, despite similar $V_a$ of S1103Y and S524Y compared to WT, the slope factor was larger for the former and smaller for the latter when compared to WT. The slope of the Boltzmann curve represents the voltage sensitivity that can be converted to the gating charge (Hodgkin and Huxley, 1952), (Spray et al., 1981), indicating that the voltage sensitivity can be modulated. The exposure to 10 µM AMIO did not change the voltage-dependent activation of WT and all studied polymorphisms. Interesting, S1103Y has relatively high frequency in the African-descendent population, and it was linked to a proarrhythmic phenotype in the presence of antiarrhythmic drugs that lead to QT prolongation, like AMIO (Splawski et al., 2002). Although it is not clear which splice variant was used in the referred study, this phenomenon can be associated with a stronger voltage dependency for channel activation, which is in accordance with the increased voltage sensitivity observed in our study that is further accentuated by exposure to AMIO.

Moreover, the voltage dependency for stationary inactivation was not different among all variants compared to WT, according to previous findings (Shinlapawittayatorn et al., 2011), (Plant et al., 2006), (Abe et al., 2018), while it is
not clear from those reports which splice variant was used. Tan et al., 2005 (Tan et al., 2005), however, observed no differences in $V_h$ of all polymorphisms described in Q1077del background, while S1103Y displayed a positive shift, and R1193Q a negative shift of $V_h$ (using Q1077 background). It is important to recognize that biophysical properties of $I_{Na}$ can sometimes vary depending on several experimental factors including temperature, solutions, presence of $\beta$ subunit, and pH (Plant et al., 2006), (Abe et al., 2018). Nevertheless, in our experimental conditions, which was standardized for all variants, no significant differences were observed among the $V_h$ of Nav1.5 polymorphisms. Besides, exposure to AMIO displayed a significant negative shift in $V_h$ of all variants compared to its respective value before to exposure to the drug, while with a comparable magnitude between all of them.

Block of $I_{Na}$ by AMIO has long been recognized to be dependent upon binding primarily to the closed and inactivated states of the channel (Follmer et al., 1981). This is consistent with an increased time to recovery from inactivation, as reported here. Among polymorphisms, the African-related S1103Y and the Asian-related R1193Q displayed slower kinetics for the fast-component of the recovery from inactivation, compared to WT. This observation is discrepant from previous reports in which no difference was observed for both variants (Tan et al., 2005). Nevertheless, the S1103Y and R1193Q polymorphisms are located in the DII-DIII linker and this region of Nav1.5 has been implicated in the channel inactivation (Camacho et al., 2006). Also, slower kinetics of the fast-component for recovery from inactivation was maintained after exposure to AMIO. On the other hand, AMIO leads to a substantial increasing in the time constant for the slow component of the inactivation recovery in all variants, which predictably contributes to the markedly use-dependency that is typical for this drug. Besides, the magnitude of increasing in the slow time constant is
comparable between WT and all variants, except for S524Y, which displayed a significant increase compared to the other variants exposed to AMIO.

AMIO also interfered with the fraction of the current (and probably the channel) that undergoes a fast and slow recovery from inactivation. S524Y, S1103Y, and R1193Q showed a higher fraction of the current that recovers by fast kinetics. This effect could contribute to the lower use-dependent block observed for R1193Q, particularly at more depolarized holding potential. Besides, it can strongly contribute to the reduced pharmacological potency of AMIO that is observed when compared to WT. Interestingly, in a previous study using Nav1.4 it was shown that DIV-S6 has a pivotal role in the ability of lidocaine to modulate slow recovery from sodium channel inactivation (Gawali et al., 2015).

AMIO has a markedly use dependency that happens in a combination of the stimulation frequency and the duration of the depolarizing pulses (Follmer et al., 1987), (Kodama et al., 1997). The use dependence of class I antiarrhythmic drug is a classical phenomenon providing important implications to the treatment of arrhythmic diseases. While the use-dependency of all variants was comparable to WT at more negative potentials, one variant was different when membrane holding potential was set at -80 mV. R1193Q, an Asian-related polymorphism, displayed almost no frequency dependency. Differences in membrane holding (or diastolic) potential are found across the human myocardium, as expressed for example by the more depolarized environment of the atria compared to ventricles (Li et al., 2002). Such contrast could lead to a heterogeneous profile of AMIO action. Importantly, it could interfere with AMIO effects on treating supraventricular arrhythmias, especially reentrant tachyarrhythmia or electrical disturbances associated with atrial ectopic focus, including atrial flutter and fibrillation. As discussed above, ectopic activity is
more dependent on membrane excitability and therefore, on the $I_{\text{Na}}$ (Heijman et al., 2017), (Heger et al., 1981), (Hamer et al., 1983). The clinical relevance of more accurate knowledge and prescription of AMIO to treat supraventricular arrhythmias becomes more evident with the understanding that AMIO is already used to treat atrial fibrillation and other supraventricular arrhythmias (Hamilton et al., 2020). Besides, other studies report a relatively higher efficiency of AMIO to treat atrial fibrillation when compared to other antiarrhythmic drugs (Vitolo et al., 1981), (Kochiadakis et al., 1998), while the efficiency of AMIO to restore an acute onset of atrial fibrillation can vary greatly (35-65%) (Hamilton et al., 2020), (Chevalier et al., 2003). However, additional factors probably contribute to the atrial AMIO selectivity. For example, the slower repolarization phase of atrial action potential leads to failure of the AP to restore maximum resting potential at rapid pacing rates. Also, the slower repolarization further contributes to significantly shortening of the diastolic interval in atria but not ventricles, thus reducing the rate of dissociation of sodium blockers from the channel.

Taken together our data indicate that AMIO has reduced pharmacological potency among the ethnic-related Nav1.5 polymorphisms when compared to WT, which is consistent with specific modulation of the variant's biophysical properties. In addition, R1193Q displayed a reduction in the use-dependency induced by AMIO at relative depolarization of cell membrane, comparing cells at -120mV and -80 mV. We understand that our work may guide future studies focusing on identifying the efficiency of AMIO in treating different types of arrhythmias as well as to establish more accurate and specific guidelines for its use depending on the Nav1.5 polymorphisms. However, proof of concept studies using in vivo experiments is still necessary.
Authorship Contributions

Participated in research design: Joviano-Santos, Santos-Miranda, Roman-Campos.

Conducted experiments: Joviano-Santos, Santos-Miranda, and Sarmento.

Contributed new reagents or analytic tools: Roman-Campos.

Performed data analysis: Joviano-Santos, Santos-Miranda, Sarmento, and Roman-Campos.

Wrote or contributed to the writing of the manuscript: Joviano-Santos, Santos-Miranda, Sarmento, and Roman-Campos.
References


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There is no conflict of interest
Figure Legends

**Figure 1: Ethnic-related Nav1.5 polymorphisms.** (A) Schematic representation of the ethnic-related single nucleotide mutations distribution in the Nav1.5 channel. Sequences are compared between Nav1.5 channel isoforms (B) Percentage of cells showing macroscopic voltage-dependent currents. All Nav1.5 polymorphisms are expressed in the Q1077 splice variant. \( N = (\text{WT} - 58, \text{S524Y} - 32, \text{S1103Y} - 32, \text{R1193Q} - 37, \text{V1951L} - 44) \), * \( p < 0.05 \). Chi-Square Test.

**Figure 2: Dose-response blockage of \( I_{\text{Na}} \) by amiodarone.** (A) Time course and (B) representative traces of amiodarone blockage of \( I_{\text{Na}} \) on the ethnic-related Nav1.5 polymorphisms. (C) Summarized dose-response of amiodarone and estimated concentration to reach 50% of the current block (\( IC_{50} \)). \( N = 5-11/\text{points} \). * \( p < 0.05 \). Two-way ANOVA; comparing WT and polymorphism.

**Figure 3: Comparison of the voltage-dependent activation of \( I_{\text{Na}} \) among Nav1.5 ethnic-related polymorphisms.** (A) Representative traces of the voltage-dependent activation of the current before (upper panel, black traces) and after exposure to amiodarone (lower panel, colored traces). Only first 25 traces are shown. Scale is the same within the same group (-) amiodarone and (+) amiodarone. Current and time scale values are the same for all representative traces. (B) Normalized current-voltage (IV) plots by the peak current are shown for each tested potential before (black traces) and after exposure to amiodarone (colored traces). (C) Voltage-dependent activation curves as a function of the tested membrane potential. \( V_{a}: \)
voltage in which half of the maximum conductance was achieved. \( N = (\text{WT} - 10, \text{S524Y} - 6, \text{S1103Y} - 7, \text{R1193Q} - 6, \text{V1951L} - 7) \). Two-way ANOVA comparing WT and polymorphism, seen in the Table 1 for statistics.

**Figure 4:** Comparison of the stationary voltage-dependent inactivation of \( I_{\text{Na}} \) among Nav1.5 ethnic-related polymorphisms. (A) Representative traces of the voltage-dependent activation of the current before (black traces) and (B) after exposure to amiodarone (colored traces). The orange thick traces highlight the current recorded at -80 mV conditioning pulse (C) Voltage-dependent channel availability curves as a function of the tested membrane potential. \( V_h \): voltage in which half of the maximum channel availability is achieved \( N = (\text{WT} - 11, \text{S524Y} - 7, \text{S1103Y} - 5, \text{R1193Q} - 7, \text{V1951L} - 7) \). Two-way ANOVA comparing WT and polymorphism, seen in the specific Table.

**Figure 5:** Comparison of the recovery from inactivation of \( I_{\text{Na}} \) among Nav1.5 ethnic-related polymorphisms. (A) Representative traces of \( I_{\text{Na}} \) the recovery from inactivation before (black traces) and (B) after exposure to amiodarone (colored traces). Only the initial, fast component of the recovery is shown for better visualization. (C) Fractional recovery of \( I_{\text{Na}} \) from inactivation is plotted as a function of the interval between the conditioning and test pulses. The recovery is better fitted with a two exponential function that implicates a fast and slow component of the current recovery. \( N = (\text{WT} - 11, \text{S524Y} - 7, \text{S1103Y} - 7, \text{R1193Q} - 7, \text{V1951L} - 7) \). Two-way ANOVA comparing WT and polymorphism, seen in the specific Table.
Figure 6: Use-dependent block of $I_{\text{Na}}$ by amiodarone in membrane holding potential at -120 mV. (A) Representative traces of $I_{\text{Na}}$ recorded at 1, 2, and 5 Hz and normalized by 1 Hz. Holding potential was set to -120 mV (B) summarized data of the use-dependent block. Data were linearly regressed and the slope was used to compare the use-dependent effects of amiodarone between variants. $N = \{\text{WT} - 6, \text{S524Y} - 7, \text{S1103Y} - 8, \text{R1193Q} - 8, \text{V1951L} - 8\}$. Two-way ANOVA comparing WT and polymorphism, seen in the specific Table.

Figure 7: Use-dependent block of $I_{\text{Na}}$ by amiodarone in membrane holding potential at -80 mV. (A) Representative traces of $I_{\text{Na}}$ recorded at 1, 2, and 5 Hz and normalized by 1 Hz. Holding potential was set to -80 mV (B) summarized data of the use-dependent block. Data were linearly regressed and the slope was used to compare use-dependent effects of amiodarone between variants. $N = \{\text{WT} - 6, \text{S524Y} - 7, \text{S1103Y} - 6, \text{R1193Q} - 7, \text{V1951L} - 6\}$. Two-way ANOVA comparing WT and polymorphism, seen in the specific Table.
Table 1: Voltage-dependent activation parameters of $I_{Na}$ among Nav1.5 ethnic-related polymorphisms obtained using Boltzmann equation fitted to the data.

<table>
<thead>
<tr>
<th>AMIO (-)</th>
<th>AMIO (-)</th>
<th>AMIO (+)</th>
<th>AMIO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>$V_a$ (mV)</td>
<td>Slope</td>
<td>$V_a$ (mV)</td>
</tr>
<tr>
<td>WT (n = 10)</td>
<td>4.1±0.4</td>
<td>-34.4±1.3</td>
<td>4.9±0.3</td>
</tr>
<tr>
<td>S524Y (n = 6)</td>
<td>1.9±0.1*</td>
<td>-40.1±1.1</td>
<td>2.7±0.2*</td>
</tr>
<tr>
<td>S1103Y (n = 7)</td>
<td>6.3±0.4*</td>
<td>-29.6±1.3</td>
<td>6.7±0.6</td>
</tr>
<tr>
<td>R1193Q (n = 6)</td>
<td>5.3±0.4</td>
<td>-33.4±1.7</td>
<td>6.2±0.4</td>
</tr>
<tr>
<td>V1951L (n = 7)</td>
<td>4.9±0.5</td>
<td>-33.9±2.1</td>
<td>5.5±0.3</td>
</tr>
</tbody>
</table>

$p < 0.05$, comparing polymorphism (-) AMIO to WT (-) AMIO.

$p < 0.05$ & comparing polymorphism (+) AMIO to WT (+) AMIO.

$V_a$: voltage in which half of the maximum conductance was achieved.

Kolmogorov–Smirnov test; Two-way ANOVA followed by Tukey’s post-test.
Table 2: Voltage-dependent inactivation parameters of $I_{Na}$ among Nav1.5 ethnic-related polymorphisms obtained using Boltzmann equation fitted to the data.

<table>
<thead>
<tr>
<th></th>
<th>AMIO (-)</th>
<th>AMIO (+)</th>
<th>AMIO (+)</th>
<th>AMIO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>$V_h$ (mV)</td>
<td>Slope</td>
<td>$V_h$ (mV)</td>
</tr>
<tr>
<td>WT</td>
<td>5.1±0.7</td>
<td>-75.9±3.7</td>
<td>6.4±0.5**</td>
<td>-87.3±6.3**</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S524Y</td>
<td>4.6±0.6</td>
<td>-74.9±4.1</td>
<td>5.7±0.6</td>
<td>-83.9±4.1**</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1103Y</td>
<td>5.2±0.6</td>
<td>-78.8±3.8</td>
<td>7.1±1.3**</td>
<td>-89.4±5.9**</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1193Q</td>
<td>4.7±0.6</td>
<td>-76.8±1.9</td>
<td>5.5±0.8</td>
<td>-84.6±2.3**</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1951L</td>
<td>5.1±0.5</td>
<td>-77.9±2.2</td>
<td>5.7±0.3</td>
<td>-86.3±1.2**</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$p < 0.05$ ** comparing (-) AMIO versus (+) AMIO.

$V_h$: voltage in which half of the maximum channel availability is achieved

Kolmogorov–Smirnov test; Two-way ANOVA followed by Tukey’s post-test.
Table 3: Time constant ($t_1$) of $I_{Na}$ recovery among Nav1.5 ethnic-related polymorphisms obtained using a two exponential decay equation.

<table>
<thead>
<tr>
<th>Time constant</th>
<th>AMIO (-)</th>
<th>AMIO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT</strong> (n = 11)</td>
<td>$1.91\pm0.61$ ms</td>
<td>$2.62\pm0.74$ ms</td>
</tr>
<tr>
<td><strong>S524Y</strong> (n = 7)</td>
<td>$1.53\pm0.31$ ms</td>
<td>$2.64\pm1.46$ ms</td>
</tr>
<tr>
<td><strong>S1103Y</strong> (n = 7)</td>
<td>$3.04\pm0.86^*$ ms</td>
<td>$3.66\pm1.27^{**}$ ms</td>
</tr>
<tr>
<td><strong>R1193Q</strong> (n = 7)</td>
<td>$3.12\pm0.36^*$ ms</td>
<td>$3.49\pm0.75^{**}$ ms</td>
</tr>
<tr>
<td><strong>V1951L</strong> (n = 7)</td>
<td>$2.21\pm0.41$ ms</td>
<td>$2.91\pm1.41$ ms</td>
</tr>
</tbody>
</table>

$p < 0.05$ * comparing polymorphism AMIO (-) to WT AMIO (-)

$p < 0.05$ ** comparing polymorphism AMIO (+) to WT AMIO (+)

Kolmogorov–Smirnov test; Two-way ANOVA followed by Tukey’s post-test.
### Table 4: Time constant ($\tau_2$) of $I_{Na}$ recovery among Nav1.5 ethnic-related polymorphisms obtained using a two exponential decay equation.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Time constant</th>
<th>AMIO (−)</th>
<th>AMIO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 11)</td>
<td>$\tau_2$</td>
<td>9.57±10.17</td>
<td>199.28±59.07</td>
</tr>
<tr>
<td>S524Y (n = 7)</td>
<td>$\tau_2$</td>
<td>6.85±5.79</td>
<td>290.89±86.68**</td>
</tr>
<tr>
<td>S1103Y (n = 7)</td>
<td>$\tau_2$</td>
<td>19.16±22.97</td>
<td>229.08±66.23</td>
</tr>
<tr>
<td>R1193Q (n = 7)</td>
<td>$\tau_2$</td>
<td>16.81±22.06</td>
<td>156.98±109.33</td>
</tr>
<tr>
<td>V1951L (n = 7)</td>
<td>$\tau_2$</td>
<td>7.21±7.29</td>
<td>270.61±124.86</td>
</tr>
</tbody>
</table>

$p < 0.05$ ** comparing polymorphism (+) AMIO to WT AMIO (+)

Kolmogorov–Smirnov test; Two-way ANOVA followed by Tukey’s post-test.
Table 5: Initial quantities ($A_1$) relative to the fast-component of $I_{\text{Na}}$ recovery among Nav1.5 ethnic-related polymorphisms obtained using a two exponential decay equation.

<table>
<thead>
<tr>
<th></th>
<th>AMIO (-)</th>
<th>AMIO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 11)</td>
<td>A1</td>
<td>0.78±0.15</td>
</tr>
<tr>
<td>S524Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 7)</td>
<td>A1</td>
<td>0.71±0.18</td>
</tr>
<tr>
<td>S1103Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 7)</td>
<td>A1</td>
<td>0.68±0.32</td>
</tr>
<tr>
<td>R1193Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 7)</td>
<td>A1</td>
<td>0.67±0.32</td>
</tr>
<tr>
<td>V1951L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 7)</td>
<td>A1</td>
<td>0.74±0.17</td>
</tr>
</tbody>
</table>

$p < 0.05$ ** comparing polymorphism (+) to WT AMIO (+)

Kolmogorov–Smirnov test; Two-way ANOVA followed by Tukey’s post-test.
Table 6: Initial quantities ($A_2$) relative to the slow-component of $I_{Na}$ recovery among Nav1.5 ethnic-related polymorphisms obtained using a two exponential decay equation.

<table>
<thead>
<tr>
<th></th>
<th>AMIO (-)</th>
<th>AMIO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>WT (n = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.21±0.15</td>
<td>0.35±0.16</td>
</tr>
<tr>
<td>S524Y (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.28±0.18</td>
<td>0.18±0.08**</td>
</tr>
<tr>
<td>S1103Y (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.31±0.32</td>
<td>0.19±0.08**</td>
</tr>
<tr>
<td>R1193Q (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.32±0.32</td>
<td>0.17±0.05**</td>
</tr>
<tr>
<td>V1951L (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.25±0.17</td>
<td>0.28±0.11</td>
</tr>
</tbody>
</table>

$p < 0.05$ ** comparing polymorphism (+) AMIO to WT AMIO (+)

Kolmogorov–Smirnov test; Two-way ANOVA followed by Tukey's post-test.
Table 7: Linear regression of frequency-dependent block of $I_{\text{Na}}$ among Nav1.5 ethnic-related polymorphisms, at -120 mV holding potential

<table>
<thead>
<tr>
<th></th>
<th>Pearson</th>
<th>R2</th>
<th>slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 6)</td>
<td>-0.95±0.04</td>
<td>0.91±0.09</td>
<td>-0.76±0.23</td>
</tr>
<tr>
<td>S524Y (n = 7)</td>
<td>-0.95±0.03</td>
<td>0.91±0.05</td>
<td>-0.58±0.15</td>
</tr>
<tr>
<td>S1103Y (n = 8)</td>
<td>-0.93±0.06</td>
<td>0.88±0.10</td>
<td>-0.68±0.21</td>
</tr>
<tr>
<td>R1193Q (n = 8)</td>
<td>-0.93±0.03</td>
<td>0.88±0.06</td>
<td>-0.77±0.21</td>
</tr>
<tr>
<td>V1951L (n = 8)</td>
<td>-0.97±0.16</td>
<td>0.91±0.06</td>
<td>-0.75±0.11</td>
</tr>
</tbody>
</table>

p > 0.05 comparing to WT

Kolmogorov–Smirnov test; Student T-test.
Table 8: Linear regression of frequency-dependent block of $I_{\text{Na}}$ among Nav1.5 ethnic-related polymorphisms, at -80 mV holding potential

<table>
<thead>
<tr>
<th></th>
<th>Pearson</th>
<th>R2</th>
<th>slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 6)</td>
<td>-0.91±0.08</td>
<td>0.84±0.15</td>
<td>-0.32±0.24</td>
</tr>
<tr>
<td>S524Y (n = 7)</td>
<td>-0.30±0.82</td>
<td>0.67±0.32</td>
<td>-0.14±0.30</td>
</tr>
<tr>
<td>S1103Y (n = 6)</td>
<td>-0.79±0.17</td>
<td>0.65±0.27</td>
<td>-0.22±0.14</td>
</tr>
<tr>
<td>R1193Q (n = 7)</td>
<td>0.22±0.91*</td>
<td>0.76±0.27</td>
<td>-0.015±0.18*</td>
</tr>
<tr>
<td>V1951L (n = 8)</td>
<td>-0.91±0.11</td>
<td>0.84±0.19</td>
<td>-0.45±0.17</td>
</tr>
</tbody>
</table>

p < 0.05 * comparing to WT

Kolmogorov–Smirnov test; Student T-test.
Figure 1

A

B

Cells with macroscopic $I_{\text{Na}}$ (%)
Figure 3

A

2 nA | 4 ms

WT

WT+AMIO

S524Y

2 nA | 4 ms

S524Y+AMIO

S1103Y

2 nA | 4 ms

S1103Y+AMIO

R1193Q

2 nA | 4 ms

R1193Q+AMIO

V1951L

2 nA | 4 ms

V1951L+AMIO

B

Normalized current (%)

C

G/G_{MAX}

V_a = -36.7

V_a = -34.4

V_a = -39.7

V_a = -40.1

V_a = -33.7

V_a = -29.6

V_a = -34.5

V_a = -33.4

V_a = -33.1

V_a = -33.8

V_m (mV)

V_m (mV)
Figure 7

A

WT

5 Hz

2 Hz

1 Hz

S524Y

S1103Y

R1193Q

V1951L

B

Normalized current (%)

R² = 0.89

R² = 0.77

R² = 0.89

R² = 0.86

R² = 0.89

1 Hz

2 Hz

5 Hz

Stimulation frequency (log scaled)