# Action Potential Clamp as a Tool for Risk Stratification of Sinus Bradycardia Due to Loss-of-Function Mutations in *HCN4*

Arie O Verkerk[1,2], Ronald Wilders[1]

[1]Department of Medical Biology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands
[2]Department of Experimental Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands

## Abstract

*We performed computer simulations using the Fabbri–Severi model of a human sinus node cell to assess whether action potential (AP) clamp experiments on transfected cells could serve as a useful tool for risk stratification of sinus bradycardia due to loss-of-function mutations in the HCN4 gene, which encodes the ion channels carrying the hyperpolarization-activated 'funny' current ($I_f$).*

*For a total of 12 well-documented HCN4 mutations from the literature, we simulated AP clamp experiments on transfected cells and computed the charge carried by the wild-type or mutant $I_f$ channels during the diastolic depolarization ($Q_f$). For each of the mutations tested, we then incorporated the mutation-induced changes in fully-activated conductance and kinetics of $I_f$ into the Fabbri–Severi model and determined the cycle length in the presence of the specific mutation at different levels of autonomic tone. At each level, the beating rate of the model cell showed a close correlation with the charge carried by the HCN4 channels in the simulated AP clamp experiments ($R^2$ of 0.90–0.99). More importantly, the clinically observed minimum or resting heart rates showed a strong correlation with $Q_f$ ($R^2 = 0.73$ and $R^2 = 0.71$, respectively).*

*We conclude that AP clamp on transfected cells is a promising tool for risk stratification of sinus bradycardia due to loss-of-function mutations in HCN4.*

## 1. Introduction

The *HCN4* gene encodes the HCN4 protein, which is the major HCN isoform of the ion channels in the human sinoatrial node (SAN) that mediate the hyperpolarization-activated 'funny' current $I_f$ (also called 'pacemaker current'). $I_f$ is a depolarizing inward current during the diastolic depolarization phase of the human SAN action potential (AP) and plays an important modulatory role [1]. Not surprisingly, loss-of-function mutations in *HCN4* have been associated with familial sinus bradycardia. In the present study, we tested whether AP clamp experiments on *HCN4*-transfected cells can explain the severity of the sinus bradycardia without the need for further voltage clamp experiments and consequential data analysis to determine individual biophysical parameters of $I_f$.

First, we selected from the literature those loss-of-function mutations in *HCN4* that have been associated with familial sinus bradycardia and for which both clinical and in vitro data are available, with the condition that the clinical data include quantitative heart rate data from at least two mutation carriers. The in vitro data (decrease in fully-activated conductance, hyperpolarizing shift in voltage dependence of both steady-state activation and (de)activation time constant) were then used to determine the charge carried by $I_f$ during the diastolic depolarization phase of a prerecorded human SAN AP waveform ($Q_f$). Next, we assessed the extent to which this $Q_f$ predicts (1) the beating rate of the Fabbri–Severi model of a human SAN pacemaker cell [2] when applying the mutation-induced changes in $I_f$ observed in vitro, and (2) the heart rate of patients carrying the specific mutation in *HCN4* observed in vivo. We show that the beating rate of the model cell as well as the clinically observed minimum or resting heart rate show a strong correlation with $Q_f$ and conclude that the AP clamp is a promising tool for risk stratification of sinus bradycardia due to loss-of-function mutations in *HCN4*. Recently, the results of our study have been published as a full-length paper elsewhere [3].

## 2. Methods

### 2.1. Simulation of AP clamp experiments

A typical prerecorded AP waveform from a small and unique set of single isolated human SAN cells [4], with a cycle length of 813 ms, was used to construct a train of 100 APs that could be used as a command signal of ≈82 s duration under voltage clamp conditions, which was long enough to achieve stable behaviour of the HCN4 current during the simulated AP clamp experiments. The custom

software to simulate such AP clamp experiments was compiled as a 32-bit Windows application using Intel Visual Fortran Composer XE 2013 and run on an Intel Core i7 processor-based workstation. For the numerical reconstruction of the HCN4 current, we used the equations that we based on our experimental data on $I_f$ from the same set of single isolated human SAN cells [5]. These equations were also used by Fabbri et al. [2] in their Fabbri–Severi model of a human SAN pacemaker cell.

## 2.2. Electrical activity of human SAN cells

The electrical activity of a single isolated human SAN pacemaker cell was simulated using the comprehensive model of such a cell developed by Fabbri et al. [2], known as the Fabbri–Severi model, with updated equations for the slow delayed rectifier potassium current ($I_{Ks}$) [6]. Vagal tone was simulated by setting the model concentration of acetylcholine (ACh) to 20 nM.

The CellML code [7] of the Fabbri–Severi model, as available from the CellML Model Repository [8], was edited and run in version 0.9.31.1409 of the Windows-based Cellular Open Resource (COR) environment [9]. All simulations were run for a period of 100 s, which was long enough to achieve stable behaviour. The data analyzed are from the final five seconds of this 100 s period.

## 2.3 Loss-of-function mutations in *HCN4*

A total of 12 loss-of-function mutations in *HCN4* met the inclusion criteria described in Section 1. These 12 mutations (all heterozygous) are listed in Table 1 below, together with (1) the associated scaling factor to describe the experimentally observed decrease in fully-activated conductance ($g_f$) and (2) the hyperpolarizing shift in the voltage dependence of the steady-state activation and the time constant of (de)activation. The G482R mutation appears twice (labelled 'a' and 'b') because of widely differing in vitro data. The sources of the in vitro data and the translation of these data into the parameters in Table 1 have been described in detail elsewhere [3].

## 3. Results

Using the parameter settings of Table 1, we simulated AP clamp experiments to determine the charge that is carried by $I_f$ during the diastolic depolarization of a human SAN pacemaker cell ($Q_f$) for each of the mutations listed. Figure 1, A and B, shows the prerecorded human SAN AP with its diastolic depolarization and the associated wild-type (WT) $I_f$, which contributes to diastolic depolarization as an inward current that carries a charge of 1.00 pC (Fig. 1B, filled area). Simulating the (heterozygous) R375C mutation by halving $g_f$ and shifting the voltage dependence by −14 mV (Table 1) resulted in the $I_f$ trace of Fig. 1C,

Table 1. Mutation-induced changes in $I_f$ characteristics.

| Mutation in *HCN4* | Scaling factor for $g_f$ | Shift in voltage dependence (mV) |
|---|---|---|
| R375C | 0.5 | −14 |
| R378C | 0.43 | −7.9 |
| A414G | 1 | −19.9 ($y_\infty$), −11.9 ($\tau_y$) |
| G480R | 0.46 | −10 |
| Y481H | 1 | −44 |
| G482R[a] | 1 | −39 |
| G482R[b] | 0.35 | 0 |
| A485V | 0.32 | −15 |
| K530N | 1 | −14 |
| R550C | 1 | −4 |
| R666Q | 0.46 | 0 |
| S672R | 1 | −4.9 |
| 695X | 1 | −10.9 |

$I_f$: hyperpolarization-activated 'funny' current; $g_f$: fully-activated $I_f$ conductance; $y_\infty$: steady-state activation; $\tau_y$: time constant of (de)activation. See also Section 2.3.

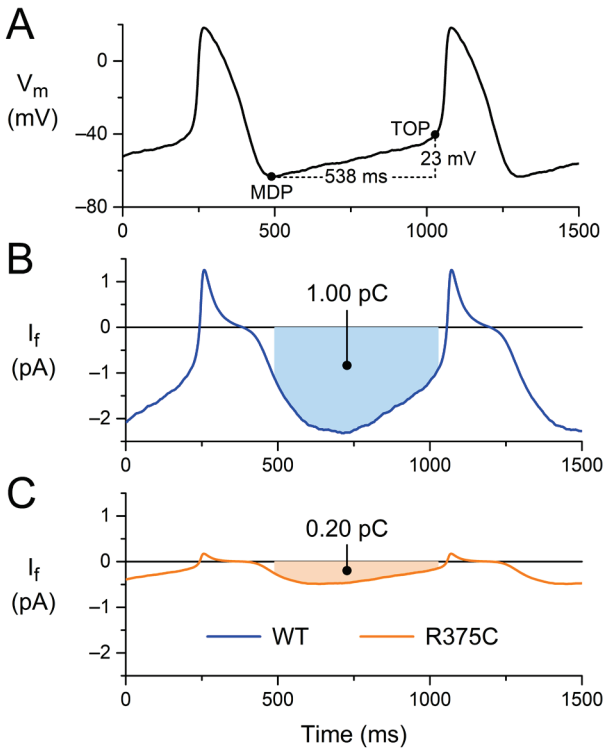


Figure 1. Simulated AP clamp experiment to determine the charge carried by $I_f$ during diastolic depolarization. (A) AP waveform of an isolated human SAN pacemaker cell. During the diastolic depolarization from the maximum diastolic potential (MDP) to the take-off potential (TOP), the membrane potential ($V_m$) depolarizes by 23 mV. (B) Reconstructed wild-type (WT) $I_f$, which carries a charge of 1.00 pC (filled area) as an inward current during diastolic depolarization. (C) Reconstructed (heterozygous) R375C mutant $I_f$, which carries a charge of 0.20 pC.

which is much smaller in amplitude and carries a charge of only 0.20 pC. Similarly, we reconstructed $I_f$ for each of the other (heterozygous) mutations listed in Table 1 and determined their $Q_f$.

We also used the parameter settings from Table 1 to run the Fabbri–Severi model and obtain its cycle length (and pacing rate) for each of the mutations, as illustrated in Figure 2 for the R375C mutation under control conditions (no rate modulation; Fig. 2A) and under vagal tone (20 nM ACh; Fig. 2B). The mutation-induced decrease in $I_f$ reduced the pacing rate from 70.2 to 56.9 beats/min under control conditions (Fig. 2A) and from 42.4 to 29.3 beats/min under vagal tone (Fig. 2B).

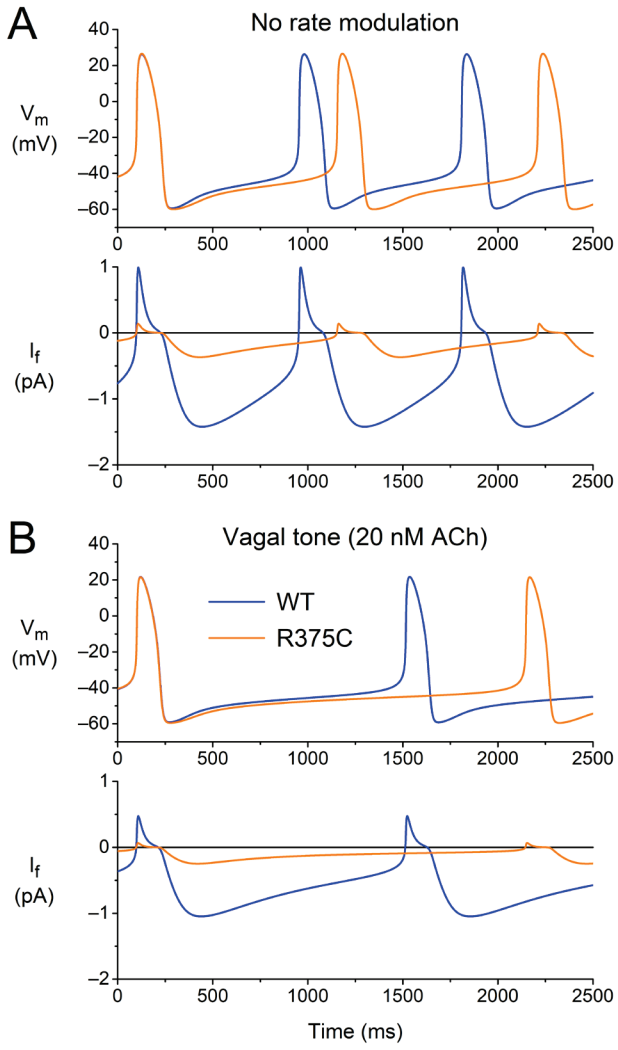


Figure 2. Electrical activity of the Fabbri–Severi model of a human SAN pacemaker cell with its default 'wild-type' $I_f$ (WT; blue lines) and with the (heterozygous) R375C mutant $I_f$ (R375C; orange lines) at different levels of autonomic tone. (A) No rate modulation (default model). (B) Vagal tone (simulated ACh concentration of 20 nM).

After obtaining pacing rate data for each of the mutations, as illustrated in Figure 2 for R375C, we plotted these pacing rates against the associated $Q_f$ from the simulated AP clamp experiments (Fig. 1), resulting in Figure 3. At both levels of autonomic tone, there is a very strong correlation between pacing rate and $Q_f$, suggesting that data on $Q_f$ obtained in AP clamp experiments on transfected cells expressing the HCN4 channels of interest, as compared to $Q_f$ for wild-type channels, can predict the severity of sinus bradycardia in mutation carriers.

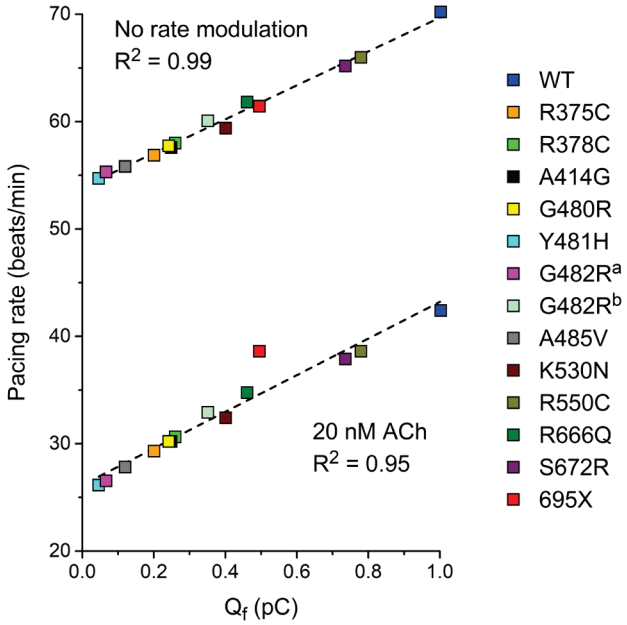


Figure 3. Pacing rate of the Fabbri–Severi model of a human SAN pacemaker cell with its default 'wild-type' (WT) $I_f$ (blue squares) and for each of the (heterozygous) mutations listed in Table 1, as a function of their $Q_f$ at different levels of autonomic tone. Dashed lines are linear fits.

Keeping in mind that the promising data presented in Figure 3 are based on the ideal case of an $I_f$ in a (simulated) human SAN pacemaker cell with biophysical parameters that are completely identical to those of the HCN4 current in an expression system, we assessed to what extent the data on $Q_f$ obtained from the (simulated) AP clamp experiments can predict the clinically observed heart rates of mutation carriers, as available in the literature for the 12 loss-of-function mutations of Table 1. For some of these mutations, heart rate data are available from 24-hour Holter recordings, for others from exercise testing, and for several from both. Figure 4A shows the minimum and average heart rates obtained during 24-hour Holter recordings, whereas resting heart rates obtained during exercise testing are shown in Figure 4B.

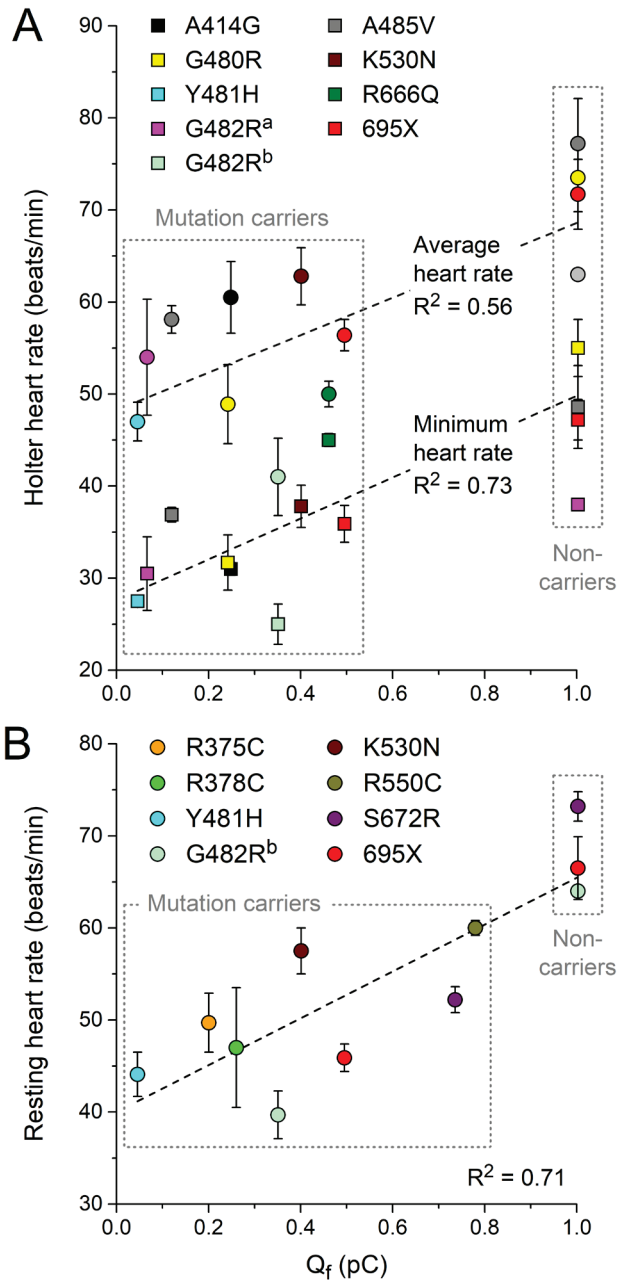


Figure 4. (A) Minimum and average heart rates obtained during 24-hour Holter recordings and (B) resting heart rates obtained during exercise testing from heterozygous carriers of the (heterozygous) mutations in *HCN4* as indicated or from non-carriers of the same family as a function of $Q_f$. Dashed lines are linear fits.

The clinically observed minimum heart rate obtained during 24-hour Holter recordings (Fig. 4A) shows a strong correlation with $Q_f$ ($R^2 = 0.73$; $p < 0.001$, ANOVA). The average heart rate shows a less clear relationship ($R^2 = 0.56$), but is still statistically significant ($p = 0.002$). The resting heart rate obtained during exercise testing (Fig. 4B) shows a strong correlation with $Q_f$ ($R^2 = 0.71$; $p = 0.001$).

## 4. Conclusion

While a translational perspective remains to be seen, we conclude that AP clamp on transfected cells, without the need for further voltage clamp experiments and data analysis to determine individual biophysical parameters of $I_f$, is a promising tool for risk stratification of sinus bradycardia due to loss-of-function mutations in *HCN4*.

## References


[1] M. Hoekstra, A. C. G. van Ginneken, R. Wilders, and A. O. Verkerk, "HCN4 current during human sinoatrial node-like action potentials," *Prog. Biophys. Mol. Biol.*, vol. 166, pp. 105–118, Nov. 2021.

[2] A. Fabbri, M. Fantini, R. Wilders, and S. Severi, "Computational analysis of the human sinus node action potential: Model development and effects of mutations," *J. Physiol.*, vol. 595, no. 7, pp. 2365–2396, Apr. 2017.

[3] A. O. Verkerk, and R. Wilders, "The action potential clamp technique as a tool for risk stratification of sinus bradycardia due to loss-of-function mutations in *HCN4*: An in silico exploration based on in vitro and in vivo data," *Biomedicines*, vol. 11, no. 9, art. no. 2447, Sep. 2023.

[4] A. O. Verkerk, R. Wilders, M. M. G. J. van Borren, R. J. G. Peters, E. Broekhuis, K. Lam, R. Coronel, J. M. T. de Bakker, and H. L. Tan, "Pacemaker current ($I_f$) in the human sinoatrial node," *Eur. Heart J.*, vol. 28, no. 20, pp. 2472–2478, Oct. 2007.

[5] A. O. Verkerk, M. M. G. J. van Borren, R. J. G. Peters, E. Broekhuis, K. Lam, R. Coronel, J. M. T. de Bakker, H. L. Tan, and R. Wilders, "Single cells isolated from human sinoatrial node: Action potentials and numerical reconstruction of pacemaker current," *Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.*, vol. 2007, pp. 904–907, Aug. 2007.

[6] A. O. Verkerk, and R. Wilders, "Human sinoatrial node pacemaker activity: Role of the slow component of the delayed rectifier $K^+$ current, $I_{Ks}$," *Int. J. Mol. Sci.*, vol. 24, no. 8, art. no. 7264, Apr. 2023.

[7] A. A. Cuellar, C. M. Lloyd, P. F. Nielsen, D. P. Bullivant, D. P. Nickerson, and P. J. Hunter, "An overview of CellML 1.1, a biological model description language," *Simulation*, vol. 79, no. 12, pp. 740–747, Dec. 2003.

[8] C. M. Lloyd, J. R. Lawson, P. J. Hunter, and P. F. Nielsen, "The CellML Model Repository," *Bioinformatics*, vol. 24, no. 18, pp. 2122–2123, Sep. 2008.

[9] A. Garny, P. Kohl, and D. Noble, "Cellular Open Resource (COR): A public CellML based environment for modelling biological function," *Int. J. Bifurcat. Chaos*, vol. 13, no. 12, pp. 3579–3590, Dec. 2003.



Address for correspondence:

Ronald Wilders, PhD
Department of Medical Biology
Amsterdam University Medical Centers
University of Amsterdam
Meibergdreef 15, 1105 AZ Amsterdam
The Netherlands
E-mail: r.wilders@amsterdamumc.nl