Diversity in cardiac sodium channel disease phenotype in transgenic mice carrying a single *SCN5A* mutation

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Lethal ventricular arrhythmias are increasingly considered an important cause of sudden death in relatively young individuals. A genetic predisposition has been recognised in many cases, and research in the last decade has focused on underlying inherited mutations in cardiac ion channels.¹ Mutations in the gene encoding the cardiac sodium channel (*SCN5A*) have been implicated in multiple arrhythmia syndromes, including long-QT syndrome (LQT3), Brugada syndrome, conduction disease, atrial standstill and sick sinus syndrome.²⁻⁵ Although caused by mutations in the same ion channel, these clinical syndromes each display distinct phenotypic characteristics. In LQT3, patients experience cardiac death at rest or during sleep, and apart from QT prolongation, no signs of conduction slowing are observed on the ECG.⁶ Brugada syndrome on the other hand not only manifests by ST-segment elevation in the right precordial leads, but in most cases signs of conduction disease are also present on the ECG, e.g. prolonged PQ and QRS duration in the presence of a normal QT interval.⁷ In rare cases, one single *SCN5A* mutation can result in multiple rhythm

disturbances within one family. The first such mutation, the *SCN5A*-1795insD mutation, was characterised by our group in a large multigenerational family which came to the attention of cardiologists in the north of the Netherlands (Groningen) in 1958.⁸ In this family extensive variability in type and severity of symptoms was documented, including ECG features of sinus node dysfunction, bradycardia, conduction disease, Brugada syndrome (ST-segment elevation), and long-QT syndrome (QT-interval prolongation), either in isolation or in combinations thereof.^{8,9} Therefore, an intriguing clinical and genetic overlap exists between these arrhythmia syndromes, previously considered separate entities. Similar findings have subsequently been reported for other *SCN5A* mutations.¹⁰⁻¹²

From a biophysical point of view, a wide range of *SCN5A* mutations can lead to multiple rhythm disturbances by modulating various gating properties of the sodium channel.¹³ Mutations disrupting fast inactivation of the sodium current cause a persistent inward current, resulting in delayed repolarisation and QT prolongation (gain of function mutations).¹⁴ In contrast, loss of function mutations invariably reduce the total amount of available sodium current, as is thought to underlie Brugada syndrome and conduction disease.² Therefore, the simultaneous presence of long-QT syndrome and Brugada syndrome or conduction disease in one patient was considered improbable prior to our description of the 1795insD family. Expression studies of the 1795insD mutation in *Xenopus* oocytes revealed the presence of reduced sodium current density, with a negative shift in voltage dependence of inactivation, but no persistent inward current.⁸ When expressed in human embryonic kidney (HEK293) cells, the mutation caused a disruption of fast inactivation resulting in a sustained inward sodium current, in addition to an increased slow inactivation leading to decreased sodium channel availability under conditions mimicking fast heart rates.¹⁵ By introducing these characteristics in a computational simulation model of a ventricular myocyte, it was shown that the 1795insD mutation can indeed cause both LQT and Brugada syndrome depending on the existing heart rate.¹⁶

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Figure 1. (A) ECG analysis displaying prolongation of PQ, QRS, and QTc intervals, and more severe bradycardia episodes in *Scn5a*^{1798insD/+} mice. (B) Activation maps from epicardial mapping experiments indicating conduction slowing in the right ventricle of *Scn5a*^{1798insD/+} mice (redrawn from Remme et al.)¹⁷

To enable investigation of this unique mutation in vivo and at multicellular and single-cell level in the native cardiac myocyte environment, we generated transgenic mice carrying the heterozygous *Scn5a*-1798insD mutation, equivalent to human *SCN5A*-1795insD. Characterisation of this mouse model showed that it faithfully recapitulates the phenotype observed in the family.¹⁷ Mutant (*Scn5a*^{1798insD/+}) mice showed signs of sinus node dysfunction, with bradycardia and pauses lasting longer than 1000 ms, in addition to prolonged PQ, QRS and QTc intervals on surface ECGs (figure 1A). Epicardial mapping experiments on isolated hearts showed conduction slowing predominantly in the right ventricle of *Scn5a*^{1798insD/+} mice, whereas left ventricular conduction was generally unchanged (figure 1B). The predominant involvement of the right ventricle in Brugada syndrome is well documented in the clinical observation of (in)complete right bundle branch block and ST-segment elevation in the right precordial leads V₁ to V₃ in these patients. Recently, our department has shown the presence of conduction slowing in the right ventricular outflow tract together with structural changes such as hypertrophy and fibrosis in the right ventricle of an explanted heart from a patient with Brugada syndrome.¹⁸

In addition, ventricular arrhythmias have been reported to be more easily induced from the right ventricle (particularly the right ventricular outflow tract) in Brugada patients.¹⁹ As yet, the exact mechanism for this predominant role for the right ventricle remains unclear.

To study the biophysical properties of the *Scn5a*^{1798insD/+} mutation in its native environment, we performed patch-clamp measurements on isolated cardiomyocytes. In *Scn5a*^{1798insD/+} myocytes, action potential (AP) duration was prolonged compared with wildtype, and AP prolongation was most pronounced at slow pacing rates (figure 2A/B). Conversely, AP upstroke velocity as a measure of sodium channel availability was more decreased at higher stimulation frequencies in *Scn5a*^{1798insD/+} myocytes. These results confirm the proposed hypothesis of a heart rate-dependent co-existence of both LQT3 and Brugada syndromes.¹⁵ In *Scn5a*^{1798insD/+} myocytes, a drastic reduction in peak sodium current density was found (figure 2C), underlying the observed conduction slowing in mice with the mutation. A small but relevant persistent inward sodium current was measured in myocytes from *Scn5a*^{1798insD/+} mice, explaining the presence of increased AP duration and QTc prolongation. Disruption of fast inactivation of the

Figure 2. (A) Action potential prolongation measured in isolated myocytes from *Scn5a*^{1798insD/+} mice. (B) Action potential prolongation was most pronounced at slow pacing rates (left), whereas action potential upstroke velocity as a measure of sodium channel availability was more decreased at higher stimulation frequencies in *Scn5a*^{1798insD/+} myocytes (right). (C) *Scn5a*^{1798insD/+} myocytes displayed a drastic reduction in peak sodium current (redrawn from Remme et al.)¹⁷

sodium current, as evidenced by a delayed time course of sodium current decay in *Scn5a*^{1798insD/+} myocytes, potentially underlies the observed persistent current. In contrast to previous transfection studies, no other changes in kinetic properties of the sodium current (such as voltage dependence of activation or inactivation) were observed. However, these results represent the actual situation within the myocyte and thus reflect the danger of over-interpretation of electrophysiological properties attributed to ion channel mutations obtained from studies in heterologous expression systems.

In conclusion, transgenic mice carrying the *Scn5a*^{1798insD/+} mutation display a phenotype similar to patients carrying the human equivalent mutation *SCN5A*-1795insD^{-/-}, including bradycardia, right ventricular conduction delay and QT prolongation. Consequently, for the first time we have generated a mouse model which recapitulates the diverse clinical symptoms of the multiple rhythm disturbances associated with this unique mutation, including Brugada syndrome, long-QT3 syndrome and conduction disease. Therefore, the presence of a single *SCN5A* mutation is indeed sufficient to cause an overlap syndrome of cardiac sodium channel disease. Our transgenic mouse model thus represents

an excellent tool for studying the complex mechanisms underlying multiple arrhythmia syndromes associated with inherited sodium channelopathies, as well as more commonly observed acquired arrhythmias in which loss of sodium channel function is implicated, such as during myocardial ischaemia and infarction. Future studies implementing this mouse model will focus on genetic and environmental factors modifying cardiac sodium channel disease.

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