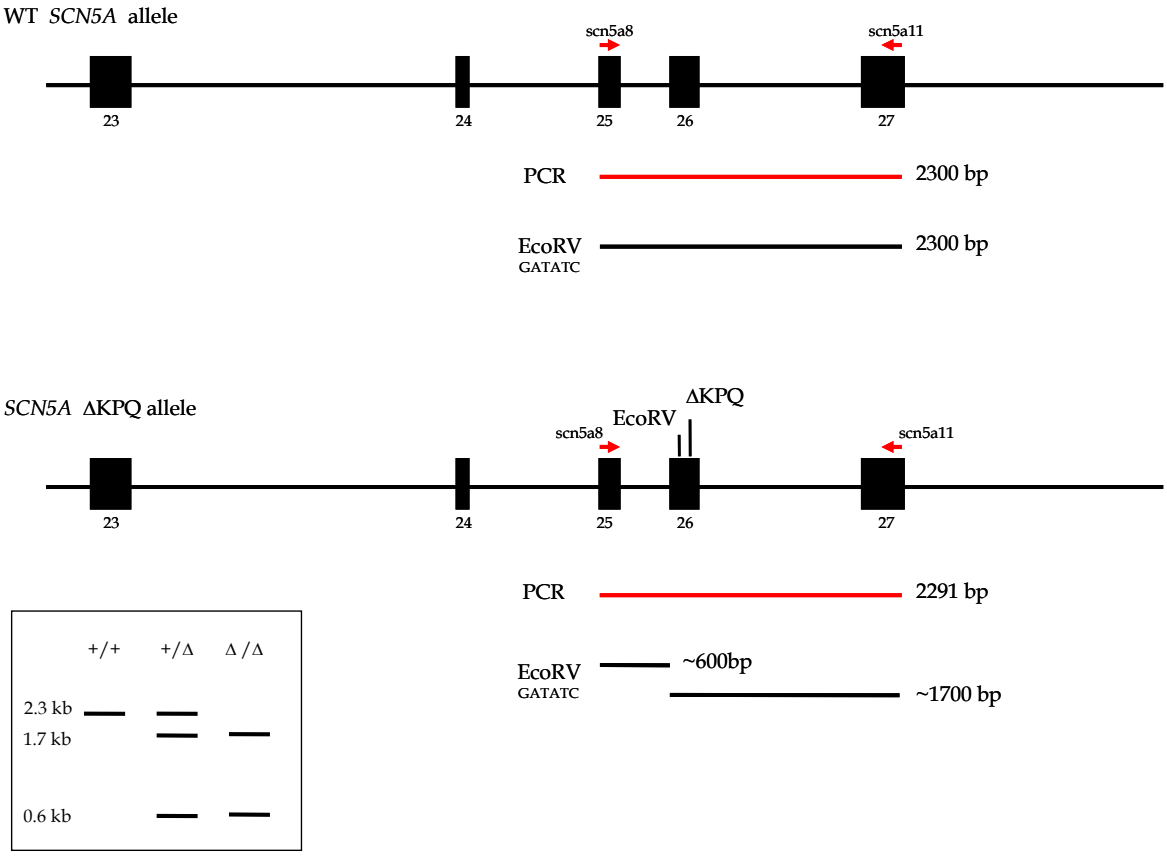
1. Supplemental Methods

1.1 Genotyping

Genomic DNA was purified by phenol/chloroform extraction, precipitated by the addition of 1/10 volume of 3M NaAc pH 6, washed and dissolved in 50 µl sterile distilled water.

As described in (Nuyens et al; Nat Med. 2001 Sep;7(9):1021-7), the mutated *SCN5A* allele differs from the WT by a deletion of 9 bp (encoding KPQ) and by the introduction of a silent *Eco*RV site upstream from the ΔKPQ deletion, in the exon 26. Similar to as described in the thesis of Dieter Nuyens, 460 ng genomic DNA were amplified with the primers scn5a8 (5´gcagtgggagacaacctctacatg 3´) and scn5a11 (5´cagctgttggtgaagtaatagtgg 3´) using Illustra PuReTaq Ready-To-Go PCR kit (GE healthcare #27-9557-01). The 2.3 kb PCR products were digested with the restriction enzyme *Eco*RV (NEB) at 37°C for at least 2h. *Eco*RV digestion results in an uncutted 2.3 kb fragment for the WT allele, and a 1.7 kb plus a 626 bp fragments for the mutated *SCN5A* ΔKPQ allele. The fragments were separated and visualized on 2% agarose gels.

Supplemental Figure 1: Scheme of ΔKPQ deletion and genotyping



1. **Supplemental Methods cont.**

1. 2 Telemetry

After surgery, animals were allowed to regain weight and were undisturbed except for weighing. After animals had regained weight after 10-14d, stress tests were limited to one test per day. In order to save in vivo animals, identical drug protocols were repeated a maximum of three times per animal with a rest period of more than 24 h in between challenges.

Analysis of PQ and QT duration could not be performed on all telemetry ECG traces as the tracings had to be completely free of noise and artefacts for these detailed measurements that require identification of the onset of the P wave and the end of the T wave. RR intervals could be readily measured in all recordings.

For flecainide therapy, the drinking water was sweetened to make up for the slightly bitter taste of flecainide. Chronic therapy with 45 mg/kg BW flecainide resulted in drug plasma levels within human therapeutic range (509 ± 110 ng/ml) as indicated in the manuscript. We performed drug dosing studies to achieve these therapeutic plasma levels. Lower doses of flecainide (15 or 30 mg/kg/d´) did not regularly result in therapeutic plasma levels

1.3 Sodium current in isolated cardiomyocytes

Differences in results from peak current measurements compared to Nuyens et al. most probably result from different holding potentials, sets of bath and pipette solutions and frequency of test pulses (in our study 0.2 Hz, not 10 Hz as in Nuyens et al.). The results are in accordance with data published by Fredj et al (References 12 and 32 in main paper).

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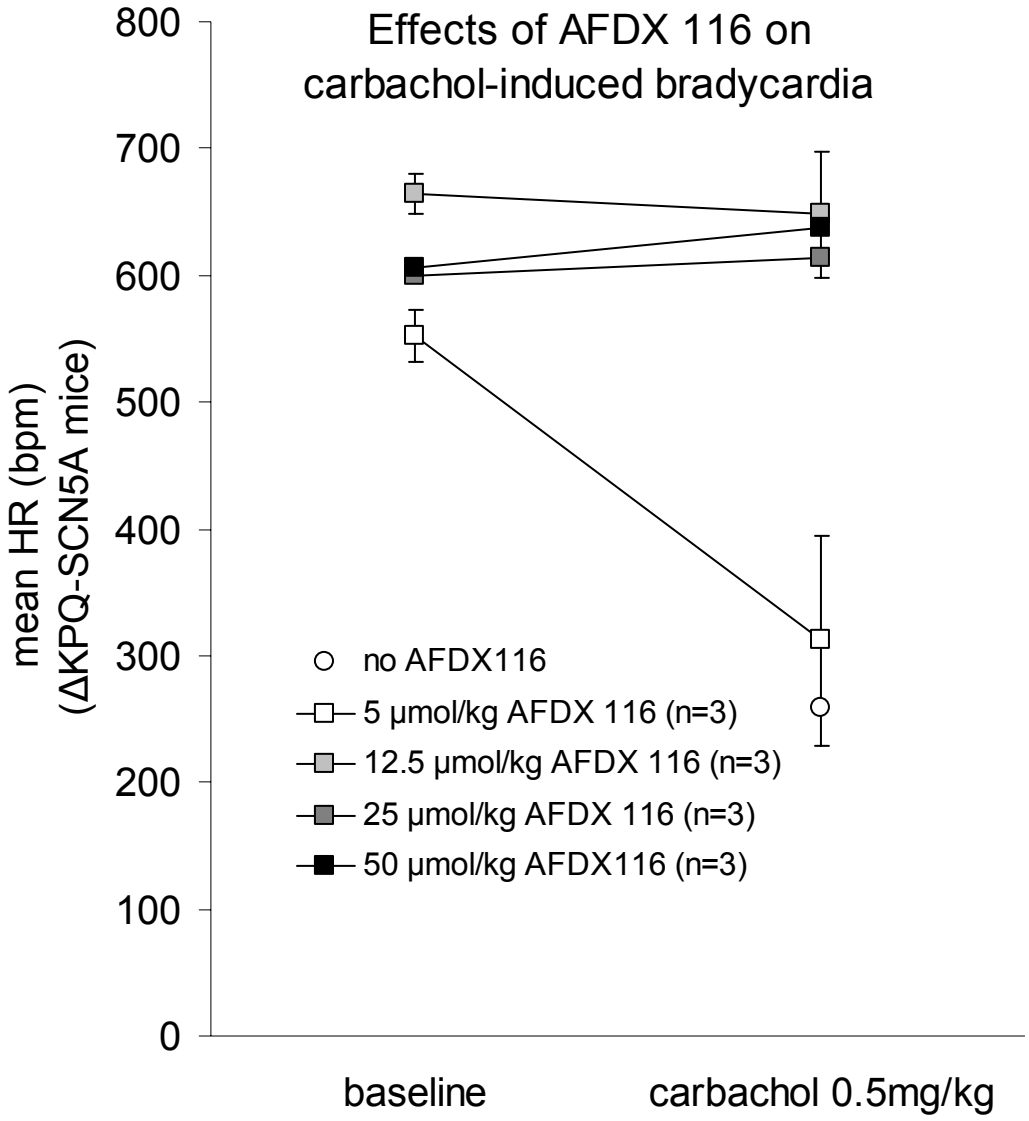
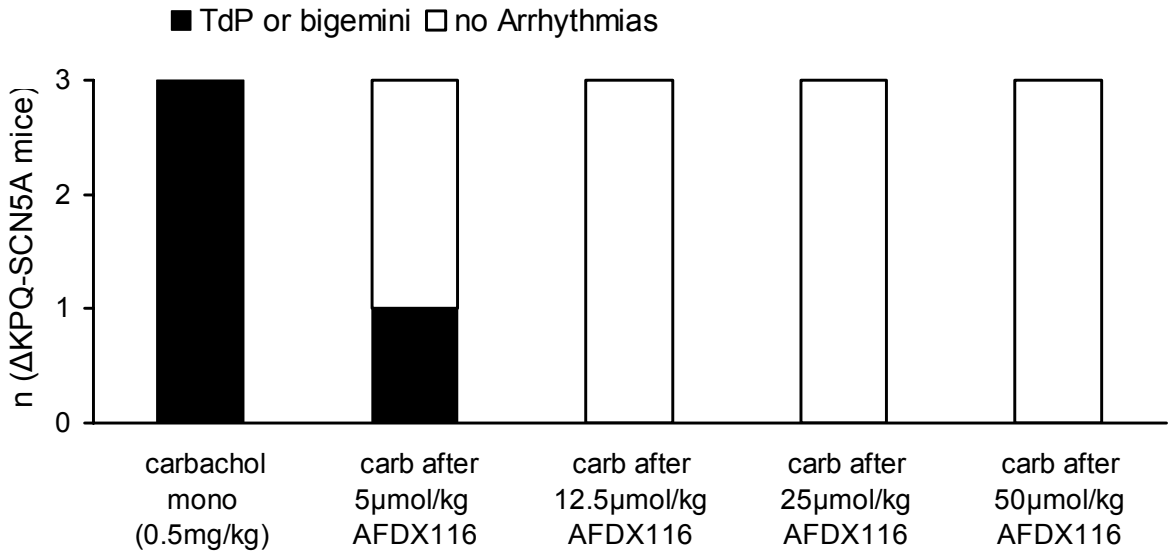
Supplemental results

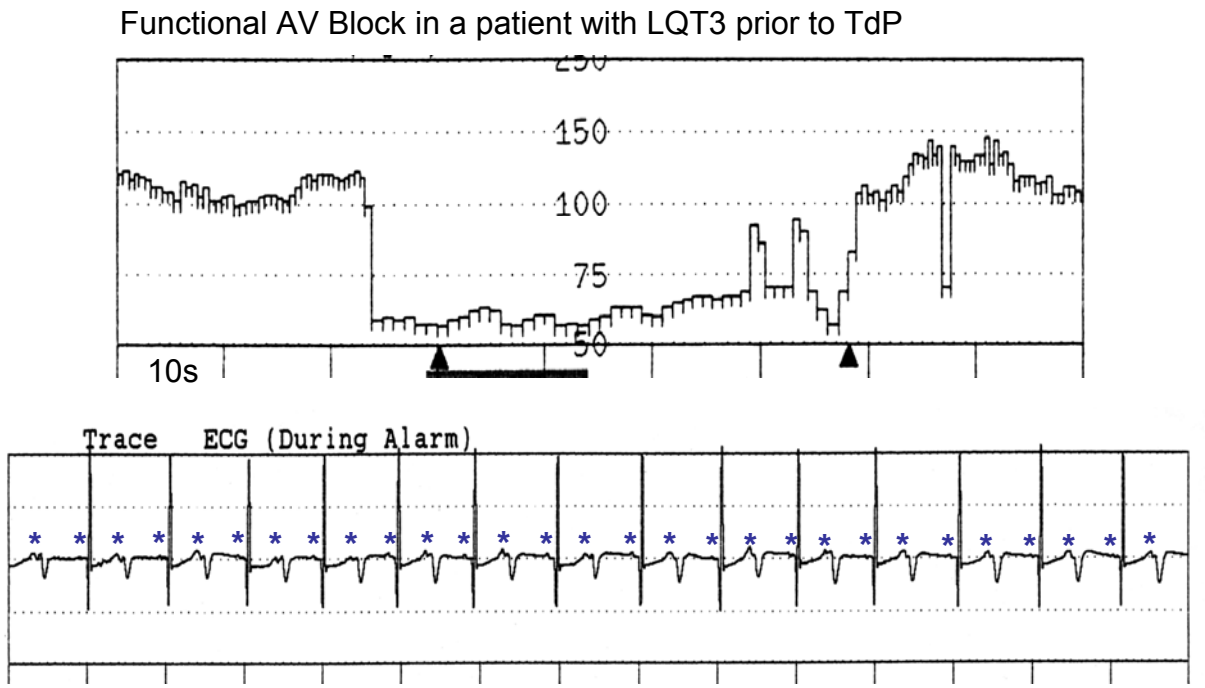
Supplemental Figure 2: Effect of muscarinic receptor blockade by AFDX116 prior to carbachol application on carbachol-induced bradycardia (above) and arrhythmias (below) in ΔKPQ-SCN5A mice. Different doses of AFDX116 were applied 15 min before application of a carbachol 0.5mg/kg BW in n=3 mice. Mice were allowed to rest for at least 72h in between applications.


Effects of AFDX 116 on
carbachol-induced bradycardia
mean HR (bpm)
(ΔKPQ-SCN5A mice)
800
700
600
500
400
300
200
100
0
no AFDX116
5 µmol/kg AFDX 116 (n=3)
12.5 µmol/kg AFDX 116 (n=3)
25 µmol/kg AFDX 116 (n=3)
50 µmol/kg AFDX116 (n=3)
baseline
carbachol 0.5mg/kg


TdP or bigemini
no Arrhythmias
n (ΔKPQ-SCN5A mice)
3
2
1
0
carbachol mono (0.5mg/kg)
carb after 5µmol/kg AFDX116
carb after 12.5µmol/kg AFDX116
carb after 25µmol/kg AFDX116
carb after 50µmol/kg AFDX116

Supplemental Figure 3

Example of bradycardia in a LQT3 patient. Home-based ECG monitor recording of an infant with long QT syndrome 3 on propranolol treatment. Transient functional 2:1 functional AV-block causes marked bradycardia in this night-time recording. Asterisks (*) mark P waves, numbers give heart rates. The infant died suddenly 2 hours later due to torsade de pointes (see Figure 2 in[4]). QT and QTc interval are markedly prolonged during bradycardia. Post-mortem genetic testing identified a missense mutation (Ala1330Pro) in the cardiac sodium channel gene SCN5A.



Supplemental Table 1

The table indicates APD 90 and 70 in both genotypes at baseline and during application of carbachol during rapid fixfrequent pacing * p<0.05 vs. WT, # p<0.05 vs. baseline. In contrast to spontaneous APD, no prolongation of APD by carbachol is seen during rapid fixfrequent pacing. (n of MAPs analyzed in brackets).

		100ms CL	120ms CL	140ms CL	150ms CL	200ms CL
	baseline	42 ms ± 3 (14)	46 ms ± 3 (15)	49 ms ± 4 (14)	49 ms ± 4 (15)	53 ms ± 5 (14)
ΔKPQ SCN5A	Carbachol 0.1µmol	37 ms ± 4 (12)	37 ms ± 3# (14)	40 ms ± 5 (10)	40 ms ± 4* (11)	41 ms ± 3 (11)
	Carbachol 1µmol	45 ms ± 2 (14)	42 ms ± 3 (15)	45 ms ± 3 (14)	42 ms ± 3 (17)	46 ms ± 4 (12)
APD 90						
	baseline	40 ms ± 2 (13)	40 ms ± 2 (13)	41 ms ± 2 (11)	42 ms ± 2 (11)	47 ms ± 3 (8)
WT	Carbachol 0.1µmol	33 ms ± 2# (12)	31 ms ± 3# (13)	32 ms ± 2# (9)	28 ms ± 2# (9)	33 ms ± 3# (10)
	Carbachol 1µmol	42 ms ± 3 (12)	42 ms ± 3 (12)	44 ms ± 4 (7)	44 ms ± 4 (7)	46 ms ± 3 (9)
	baseline	24 ms ± 2 (14)	26 ms ± 3 (15)	28 ms ± 3 (14)	28 ms ± 3 (15)	28 ms ± 4 (14)
ΔKPQ SCN5A	Carbachol 0.1µmol	21 ms ± 3 (12)	20 ms ± 2 (14)	21 ms ± 3 (10)	20 ms ± 2* (11)	22 ms ± 3* (11)
	Carbachol 1µmol	24 ms ± 2 (14)	21 ms ± 2 (15)	22 ms ± 2 (14)	20 ms ± 2 (17)	24 ms ± 3 (13)
APD70						
	baseline	23 ms ± 2 (13)	23 ms ± 2 (13)	22 ms ± 2 (11)	22 ms ± 2 (11)	27 ms ± 2 (8)
WT	Carbachol 0.1µmol	17 ms ± 1# (12)	15 ms ± 2# (13)	16 ms ± 1# (9)	14 ms ± 0# (9)	15 ms ± 2# (11)
	Carbachol 1µmol	23 ms ± 3 (12)	20 ms ± 2 (12)	19 ms ± 3 (7)	20 ms ± 3 (7)	20 ms ± 2 (9)