



Supplementary Data

Pathogenesis and drug response of iPSC-derived cardiomyocytes from two Brugada syndrome patients with different Na_v1.5-subunit mutations

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Supplementary methods

Cell index

To exclude any pharmacological effects of quinidine on viability, CardioExcyte 96 (Nanion, Germany) was used. iPSC-CMs were replated on a gelatin-coated 96-well Nanion CardioExcyte 96 sensor plate at a cell density of 50 000 cells per well. The media were changed every 2 days. Once cardiomyocytes start beating, cell impedance measurements were taken every 5 minutes for 20 seconds. When the baseline data stabilized, iPSC-CMs were incubated with different concentrations of quinidine (0, 3, 10, 30, and 100 μmol/L) for 3 hours. All data were normalized to the baseline.

Supplementary Table 1 Primers for real-time RT-PCR

| Genes | Primer sequence (5'→3') |
|-------|-------------------------|
| SCN1B | CACTCGGGCGACTACGA |
| | TCAGACACGATGGATGCC |
| SCN5A | CACATCGTGGAGCACAGC |
| | CCGTAGGCCACCCACTT |
| GAPDH | AAGGTGAAGGTCGGAGTCAAC |
| | GGGGTCATTGATGGCAACAATA |
| LMNA | AATGATCGCTTGGCGGTCTAC |
| | CACCTCTCAGACTCGGTGAT |

Supplementary Table 2 Summary of action potential parameters in iPSC-CMs

| Groups | APA (mV) | RMP (mV) | APD30 (ms) | APD50 (ms) | APD90 (ms) | V _{max} (mV/ms) | HR (bpm) |
|-----------|-------------|-------------|--------------|--------------|--------------|--------------------------|------------|
| Control 1 | 122.44±3.60 | -69.95±3.15 | 106.80±15.39 | 152.04±15.49 | 209.98±16.35 | 95.88±29.92 | 50.50±2.89 |
| Control 2 | 120.68±4.14 | -69.40±4.70 | 97.31±21.66 | 118.97±22.29 | 196.86±21.38 | 89.20±25.66 | 48.75±2.95 |
| BrS-SCN5A | 97.90±4.84 | -65.49±2.40 | 155.01±16.51 | 256.69±30.24 | 379.37±46.62 | 17.20±4.24 | 50.00±3.20 |
| BrS-SCN1B | 94.78±2.48 | -70.53±3.16 | 190.30±7.78 | 298.92±23.64 | 388.86±28.06 | 11.93±1.64 | 49.50±3.45 |

iPSC-CMs: induced pluripotent stem cell-derived cardiomyocytes; APA: action potential amplitude; RMP: resting membrane potential; APD30: action potential duration 30% repolarization; APD50: action potential duration 50% repolarization; APD90: action potential duration 90% repolarization; V_{max}: maximum upstroke velocity; HR: heart rate.

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Supplementary Table 3 Action potential parameters in iPSC-CMs modulation by different concentrations of quinidine

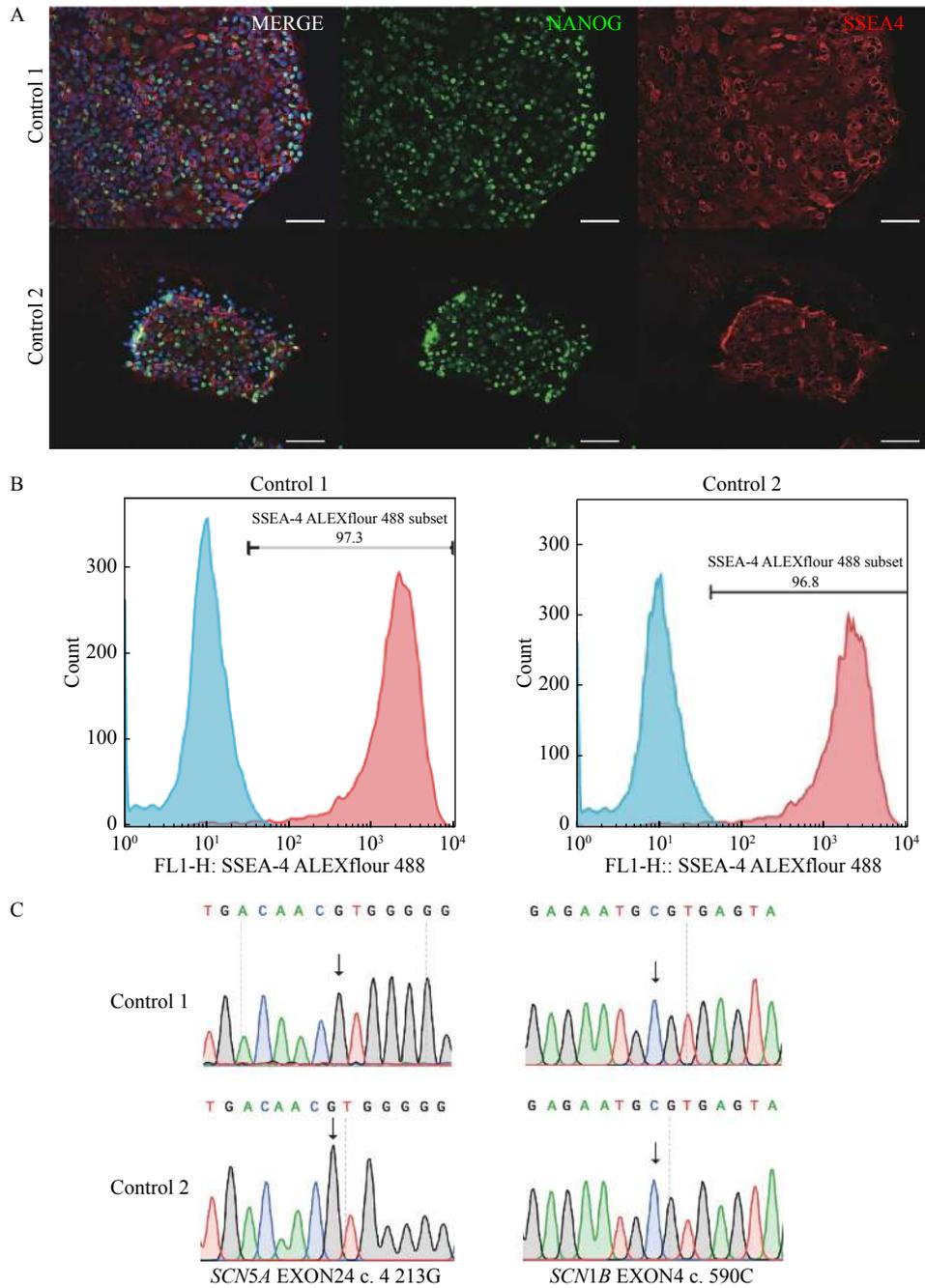
| Groups | Quinidine (μmol/L) | APA (mV) | V_{max} (mV/ms) | APD90 (ms) | APD50 (ms) | APD30 (ms) | RMP (mV) | HR (bpm) |
|-----------|--------------------|-------------|-------------------|--------------|--------------|--------------|-------------|-------------|
| Control 1 | 0 | 121.31±4.33 | 101.49±10.17 | 200.26±20.41 | 146.07±19.78 | 99.78±11.54 | -67.46±1.46 | 51.00±3.29 |
| | 0.3 | 116.46±1.85 | 84.80±5.29 | 220.57±5.13 | 144.87±6.89 | 96.79±5.05 | -65.05±1.38 | 55.15±2.87 |
| | 1.0 | 101.63±6.13 | 60.77±5.84 | 249.99±5.22 | 142.42±13.40 | 93.09±9.65 | -55.54±3.91 | 63.83±11.22 |
| | 3.0 | 72.63±9.98 | 36.28±3.48 | 290.66±20.28 | 152.71±21.59 | 96.88±13.02 | -44.43±5.51 | 60.39±9.49 |
| | 10.0 | 52.68±7.91 | 21.11±2.20 | 333.29±20.01 | 160.68±35.08 | 105.64±22.98 | -42.42±5.36 | 61.56±10.79 |
| | 30.0 | 24.26±3.17 | 8.80±0.59 | 297.18±27.96 | 143.56±33.98 | 75.33±14.09 | -31.71±1.83 | 84.45±20.96 |
| SCN5A | 0 | 98.95±3.38 | 20.8±1.08 | 290.88±13.75 | 189.06±7.15 | 146.25±7.23 | -65.06±2.28 | 50.00±6.29 |
| | 0.3 | 97.56±1.26 | 19.03±0.29 | 301.93±8.67 | 192.08±2.76 | 147.42±3.11 | -62.46±0.52 | 52.70±1.67 |
| | 1.0 | 93.41±0.48 | 17.06±0.60 | 348.47±15.64 | 213.26±2.97 | 155.61±2.49 | -58.55±1.21 | 55.40±2.50 |
| | 3.0 | 88.86±1.39 | 11.44±0.48 | 417.12±28.58 | 227.63±6.46 | 161.17±5.33 | -53.22±0.93 | 60.70±2.60 |
| | 10.0 | 73.22±2.76 | 4.13±0.25 | 428.76±40.21 | 197.38±14.74 | 133.09±11.46 | -39.69±2.53 | 75.11±12.83 |
| | 30.0 | 24.54±2.08 | 0.38±0.01 | 282.15±18.15 | 127.80±7.67 | 97.11±11.57 | -20.43±1.06 | 89.25±48.33 |
| SCN1B 1 | 0 | 93.73±2.39 | 14.46±0.43 | 397.86±25.56 | 275.46±10.57 | 206.21±9.89 | -69.52±1.36 | 47.8±5.18 |
| | 0.3 | 88.26±1.95 | 13.13±0.62 | 432.34±6.85 | 285.56±3.88 | 205.18±6.24 | -63.73±1.38 | 52.32±3.09 |
| | 1.0 | 82.88±1.40 | 11.69±1.01 | 471.46±6.18 | 271.79±9.30 | 185.93±10.06 | -54.80±1.81 | 61.68±9.99 |
| | 3.0 | 70.14±3.94 | 7.29±1.00 | 515.62±21.11 | 235.24±17.95 | 157.96±8.51 | -40.04±3.52 | 70.02±12.30 |
| | 10.0 | 50.61±2.77 | 5.46±0.70 | 498.92±28.95 | 207.70±23.92 | 145.17±16.29 | -30.59±2.30 | 74.19±12.85 |
| | 30.0 | 22.12±1.70 | 1.23±0.03 | 389.90±24.54 | 149.85±11.80 | 98.98±6.96 | -19.88±1.39 | 71.92±14.37 |

iPSC-CMs: induced pluripotent stem cell-derived cardiomyocytes; APA: action potential amplitude; RMP: resting membrane potential; APD30: action potential duration 30% repolarization; APD50: action potential duration 50% repolarization; APD90: action potential duration 90% repolarization; Vmax: maximum upstroke velocity; HR: heart rate.

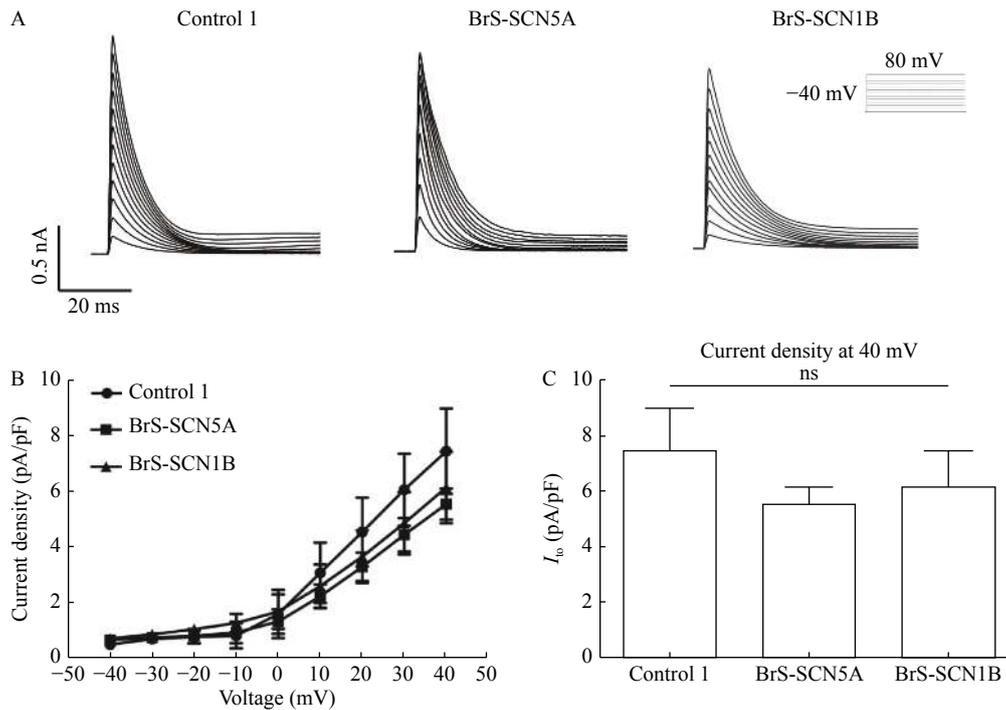
Supplementary Table 4 Review of Brugada patient-specific iPSC-CMs

| References | Variance | Sodium channel function compare to the control | AP compare to the control | Calcium handling compare to the control | Drug responses compare to the control |
|---|---|--|---|--|---|
| Elisabet Selgaa et al ^[1] | SCN5A c.1100G>A | 33.1%–45.5% of reduction in peak I_{Na} ; a positive shift in the activation $V_{1/2}$, and a negative shift in the steady-state inactivation $V_{1/2}$; faster recovery from inactivation | – | – | – |
| Ping Liang et al ^[2] | SCN5A R811H and R620H, SCN5A p.4189delT | I_{Na} reduced | V_{max} reduced; increase in peak–peak interval variability; increased burden of triggered activity | Reduced Ca^{2+} transient amplitudes; reduced maximal rising rate; more abnormal Ca^{2+} transient pattern | – |
| Ibrahim El-Batrawy et al ^[3] | SCN1B c.629T>C and c.637C>A | I_{Na} reduced; reduction of activation, enhancement of inactivation, decelerated recovery from inactivation | APA, V_{max} reduced; | More arrhythmia like events | More sensible to ajmaline and carbachol |
| Shinichiro Okata et al ^[4] | SCN5A G5349A | Increased late sodium current; no difference in peak I_{Na} , voltage-dependent activation and inactivation curves | APD90 prolonged | – | – |
| This paper | SCN5A c.4213G>A and SCN1B c.590C>T | I_{Na} reduced | APA, V_{max} reduced; prolonged APD30, APD50 and APD90 | More arrhythmia-like events; no difference in Ca^{2+} transient amplitudes | More sensible to quinidine |

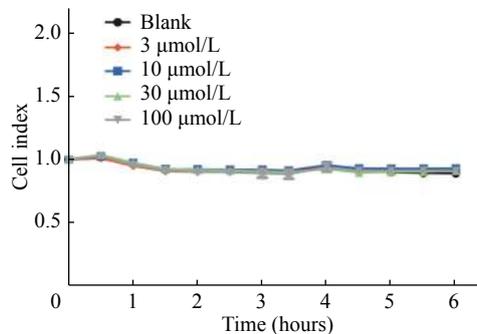
iPSC-CMs: induced pluripotent stem cell-derived cardiomyocytes; APA: action potential amplitude; RMP: resting membrane potential; APD30: action potential duration 30% repolarization; APD50: action potential duration 50% repolarization; APD90: action potential duration 90% repolarization; Vmax: maximum upstroke velocity ; HR:.



Supplementary Fig. 1 Characterization of two control iPSC lines. A: Images of iPS cells stained with pluripotency markers NANOG (green), SSEA4 (red) for Control 1 and Control 2 iPSC lines. Nuclei were stained with DAPI (blue). Scale bars represent 100 μ m/L. B: Flow cytometry analysis of pluripotency markers (SSEA4) in control iPSC lines. C: Genotyping of Control 1 and Control 2 lines focusing on the coding sequence (SCN5A c.4213G>A and SCN1B c.590C>T) confirmed by DNA sequencing.



Supplementary Fig. 2 SCN5A or SCN1B mutation showed unaffected I_{to} currents. A: Representative traces of transient outward current (I_{to}). B: Current–voltage relationships of I_{to} from Control, BrS-SCN5A, and BrS-SCN1B normalized to the cell capacitance. C: Comparison of I_{to} density at 40 mV among the control and BrS iPSC-CMs. $n=7$ for each line. Data are presented as mean \pm SEM. Comparisons between two groups were analyzed using Student's t -test. ns: no significance ($P>0.05$). iPSC-CMs: induced pluripotent stem cell-derived cardiomyocytes.



Supplementary Fig. 3 Drug effects on impedance assay measures. Cell index develop with exposure time. Data normalized to pre-drug control data. One blank with vehicle control and 4 concentrations (3, 10, 30, and 100 μ mol/L) were tested ($n=4$ for each concentration, one-way repeated-measures ANOVA). Error bars indicate SD.

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