Supplementary material

iPSC-cardiomyocyte models of Brugada syndrome – achievements, challenges and future perspectives

Aleksandra Nijak [1], Johan Saenen [2], Alain J. Labro [3,4], Dorien Schepers [1], Bart L. Loeys [1,5], and Maaike Alaerts [1,*]

[1]Center of Medical Genetics, faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, 2650 Antwerp, Belgium

[2]Department of Cardiology, University of Antwerp and Antwerp University Hospital, 2610 Antwerp, Belgium 2

[3]Department of biomedical Sciences, University of Antwerp, 2610 Antwerp, Belgium

[4]Department of Basic and Applied Medical Sciences, Ghent University, 9000 Ghent, Belgium

[5]Department of Human Genetics, Radboud University Medical Centre, 6525 Nijmegen, The Netherlands

*Correspondence: maaike.alaerts@uantwerpen.be; Tel.: +32 032759780

Supplementary Table 1: Overview of the used reprogramming and differentiation approaches, together with described genetic variants, their identification methods and connected clinical phenotypes and patient history. FB- fibroblasts; Sev – Sendai virus; Retro – retroviral vector; Lenti – lentiviral vector; Episomal – episomal vectors; ML – monolayer-based; EB – EB-based; SC – suspension culture; MxS – matrix sandwich); - G – glucose starvation; LT – lactate treatment; Puro αMHC - puromycin selection of αMHC-Puro^r containing spheres; M – male; F – female.

Reference	Reprogramming		Differentiation				Genetic diagnosis			Patient information			
	Cell type	Vector	Method	Agents	Enrichment	Culture period [days]	Gene	Variant	Method of identification	Sex	Diagnosis	Clinical phenotype	Family history
89	FB	Sev	ML	Activin A, BMP4, CHIR-90021, Xav939 in BPEL medium	N/A	28-32	SCN5A	c.4912C>T (p.R1638X)	Diagnostic screening	M	BrS	34-year old; BrS type 1 ECG and ventricular tachyarrhythmias during electrophysiological investigations	N/A
								c.468G>A (p.W156X)		M	BrS	52-year old; mild phenotype consisting of first-degree AV block and slight QRS prolongation	
68	FB	Sev	ML	CHIR99021, IWR-1	-G	40-60	SCN5A	c.2053G>A (p.R620H) and c.2626G>A (p.R811H)	Diagnostic screening	M	BrS	44 year old; unstable ventricular tachycardia after multiple episodes of recurrent syncope. BrS diagnosis based on BrS type-1 ECG pattern at rest	Sudden death in two paternal uncles and a female cousin
								c.4189delT 4190ΔA/1397 Δ(frameshift)		M	BrS	53 year old; recurrent syncope presenting as seizure-like activity. Characteristic BrS ECG changes during procainamide challenge	Sudden death in father and two paternal cousins
90	FB	Retro	ML	CHIR99021, IWR-1	LT	35-45	SCN5A	c.677C>T (p.A226V) and c.4885C>T (p.R1629X)	Genetic screening of SCN5A gene	M	BrS	21-year old, presented with syncopal events since the age of 11 during excercise. Baseline ECG with BrS type-2 pattern and first-degree atrioventricular block and QRS duration of 130 ms. Treadmill testing ECG showed a QRS widening uo to 240 ms and a BrS type-1 ECG pattern followed by polymorphic ventricular tachycardia.	Father - p.A226V with ajmaline induced type-1 ECG pattern. Mother - p.R1629X carrier asymptomatic, with baseline ECG showing first-degree AV block at 250 ms, with prolonged QRS duration of 130 ms and a saddle shaped ST elevation in V2 lead. Treadmill challenge revealed QRS prolongation up to 160 ms with no BrS characteristics. Healthy SCN5A variants negative brother and asymptomatic p.A226V carrier sister.
91	FB	Episomal	EB	END-2 conditioned media	N/A	22-31	SCN5A	c.1100G>A (p.R367H)	Familial Arrhythmia Network of Scotland	M	BrS	69-year old; classical ST-segment elevation in the ECG	N/A
			ML	CHIR99021, heparin, IWP2									

56	FB	Retro	SC ML	CHIR99021, IWP2	N/A	20-46	SCN5A	c.2204C>T (p.A735V)	N/A	N/A	BrS	BrS diagnosis in cases from four different clinical centres	One familial case with multiple mutation carriers with variable phenotypes
88	FB	Lenti	EB	END-2 cells co-culture	N/A	50	SCN5A	c.5537insTGA (p.1795insD)	Mutation analysis of SCN5A	M	LQTS3/BrS	Bradycardia, ventricular and atrial conduction slowing, including diagnosed cases of BrS and LQTS	Dutch founder mutation, identified in a large family with multiple individuals presenting variable symptoms
86	FB	Retro	EB	Wnt3a	N/A	30	SCN5A	c.5349G>A (p.E1784K)	Genetic screening of SCN5A gene	M	LQTS3/BrS	20-year old; sudden cardiac arrest while driving a car, successfully resuscitated by external defibrillator, which indicated ventricular fibrillation, while surface ECG showed QT interval prolongation. Pilsicainide administration unmasked coved-shaped ST segment elevation.	No family history of previous syncopes or significant QT interval abnormalities
69	FB	Lenti Episomal SeV	ML	CHIR99021, IWP2	LT	40-60	SCN10A	c.3749G>A (p.R1250Q) and c.3808G>A (p.R1268Q)	Genetic screening of 8 BrS-associated genes	M	BrS	SCD during ajmaline challenge, no structural abnormalities of the heart	No history of SCD
70	FB	Lenti Episomal SeV	ML	CHIR99021, IWP2	LT	40-60	SCN1B	c.629T>C (p.L210P) and c.637C>A (p.P213T)	Genetic screening of 8 BrS candidate genes	M	BrS	Syncope at rest with BrS type 1 ECG in ajmaline challenge	No history of SCD; mutations carrier son with asymptomatic BrS
71	FB	SeV	ML (MxS)	tivin A, BMP4, bF	N/A	31-35	RRAD	p.Arg211His	WES	M	BrS	Systematic ECG with typical but labile BrS type 1 ECG pattern with a history of palpitations associated with a near syncope and nocturnal agonal respiration. Treated with an ICD implantation	Identification of 4 affected relatives after flecainide challenge and one with BrS type 1 ECG pattern. No relevant symptoms observed in all of those individuals. History of unexplained SCD in probands' 41-year old uncle. RRAD variant present in all affected individuals (4) and 2 unaffected family members.
92	FB	Lenti	EB	Activin A, BMP4, bFGF, DKK1, VERGF, SB-431542	Puro αMHC	60	PKP2	R635Q (c.1904G>A)	Retrospective WES	M	BrS	31-year old; two episodes of syncope at rest with spontaneous BRS type-1 ECG. Implanted ICD with no shocks at follow up	Symptomatic grandfather with sudden death during sleep. Affected mutation-positive father with a history of syncope at rest. Mutation carrier brother with a positive flecainide test

87	FB	Lenti STEMCCA	EB	KY02111, XAV939	LT	42-56	Undefined		NGS sequencing of 12 BrS and 26 arrhythmia-related genes	M	BrS	28-years old; VF arrest at 16-years old, spontaneous BrS-type 1 ECG	Father presenting similar ECG
							Undefined			M	BrS	30-years old; ajmaline induced BrS-type 1 ECG, occasional spontaneous	N/A
							PKP2	c.302G>A (p.R101H)		M	BrS	68-years old; ajmaline induced BrS-type 1 ECG leading to VF; occasional spontaneous	N/A
55	FB	Lenti STEMCCA	ML	CHIR99021, IWP-4	LT	19	Undefined		Genetic screening of BrS-associated genes	M	BrS	42-year ols; spontaneous BrS type 1 ECG, previous story of syncope, no structural cardiac abnormalities	3 relatives with ajmaline induced BrS ECGs with no history of SCD
							Undefined			M	BrS	67-year old; spontaneous BrS type 1 ECG, previous story of syncope. Diagnosed with BrS during hospital admission for an inferior myocardial infarcion	6 relatives with ajmaline induced BrS ECGs, several family members with previous history of SCD
							CACNA1C	int19 position -7		F	BrS	24-year old; spontaneous BrS type 1 ECG, previous history of syncope, no structural cardiac abnormalities. Inducible non-sustained ventricular tachycardias in RVOT followed by ICD implantation. PR-interval prolongation (220 ms) with normal ORS- and QTc.	5 relatives with ajmaline induced BrS ECGs with no history of SCD

Suplementary Table 2: AP properties published from BrS iPSC-CM models. RMP/MDP – resting membrane potential/maximum diastolic potential; APA – action potential amplitude; APD20/APD50/APD90 – action potential duration at 20/50/90 % of depolarization; V_{max}- upstroke velocity; RT -room temperature.

Reference	Cell line	AP properties										Protocol	In sillico I_{K1} injection
		RMP/ MDP [mV]	APA [mV]	Overshoot [mV]	APD20 [ms]	APD30 [ms]	APD50 [ms]	APD90 [ms]	Vmax [v/s]	peak to peak duration [ms]	n		
89	hiPSC-CM^{WT1}	-75±1	105±5				125±20	190±20	56±12		12	Isolated cells recorded on D28-32 of differentiation. APs measured at 37°, elicited at frequency of 1Hz by 3ms	
	hiPSC-CM^{WT2}	-75±0.5	107±3				205±10	290±15	55±3		12		
	hiPSC-CM^{R1638X}	-70±0.5	100±2				160±10	205±15	18±2		14		
	hiPSC-CM^{W156X}	-70±0.5	98±5				150±20	200±20	19±2		16		
68	CON1	-58.9±0.8	106.4±1.0	48.0±0.7				443.5±17.3	15.6±0.8	49.4±5.5	10to25	APs recorded on dissociated cells at day 40-60 at 36-37 degrees	
	CON2												
	BrS1	-58.1±0.7	107.1±1.6	50.1±1.2				459.3±38.2	11.1±0.1	380.4±96.2	10to25		
	BrS2	-56.5±2.0	105.0±2.6	47.8±2.5				396.2±33.4	7.2±1.1	195.5±60.3	10to25		
	BrSp2-GE	-64.2±1.0	109.3±1.5	46.1±0.9				355.8±19.3	17.4±1.2	48.4±7.4	10to25		
90	CON1	-43.39±2.81	87.5±5.76		204.09±44.01		362.09±56.35	473.64±63.97	17.76±4.59	37.83±5.81	11	APs recorded at day 35-45 of differentiation at 34°C	Generated in real time in response to CM membrane potential; potentiometer was set to provide a standard outward current peaked at 150 pA at -75 mV
	BrS_A266V+R1629X	-49.09±6.12	91.16±8.09		277.46±48.5		430.82±60.84	493.18±62	15.76±4.59	32.57±7.92	11		
	BrS_A266V+R1629X 1.0 Hz	-57.83±0.32	73.30±1.53		92.08±5.18		166.10±4.77	221.24±8.58	10.72±0.32		3		
	BrS_A266V+R1629X 1.5 Hz	-57.87±1.54	74.12±0.93		88.61±1.82		153.60±5.87	209.77±9.63	9.21±0.09		3		
	BrS_A266V+R1629X 0.5 Hz	-60.72±2.02	69.00±1.55		95.00±4.82		164.66±9.2	216.36±12.1	14.05±1.87		3		
	BrS_A266V+R1629X 0.2 Hz	-53.52±3.17	64.10±5.35		87.37±7.43		166.30±6.97	221.25±10.31	13.21±0.02		3		
	CON1 with I_{K1} injection	-84.2±04	128.6±1.3	44.4±1.0	126.3±14.2		297.2±25	339.2±26.8	185.1±11		22		
	BrS_A266V+R1629X with I_{K1} injection	-84.4±0.2	115.6±0.9	31.3±0.9	223.8±12.3		318.2±13.9	338±14.3	41.9±5.3		45		

	CON1 with I_{K1} injection 1 Hz	-84.2±0.2	128.0±1.4	43.8±1.1	142.6±18.8		302.9±32.8	348.2±31.7	178.9±12.3		19		
	CON1 with I_{K1} injection 0.5 Hz	-84.3±0.2	129.9±1.3	45.5±1.0	186.0±32.6		381.7±53.0	426.3±52.6	193.8±13.0		19		
	CON1 with I_{K1} injection 0.2 Hz	-84.4±0.2	129.9±1.3	45.5±1.1	133.6±46.5		328.4±65.2	367.2±65.1	197.3±13.9		19		
	BrS_A266V+R1629X with I_{K1} injection non-ER 1 Hz	-84.2±0.4	115.3±0.9	31.10.9	211.7±13.4		310.9±16.1	331.5±16.6	36.9±5.7		33		
	BrS_A266V+R1629X with I_{K1} injection non-ER 0.5 Hz	-84.5±0.4	117.0±1.0	32.7±1.0	308.6±22.8		424.3±26.8	444.2±27.3	40.7±6.4		33		
	BrS_A266V+R1629X with I_{K1} injection non-ER 0.1 Hz	-84.4±0.4	114.1±1.2	29.7±1.2	284.2±29.8		396.0±35.2	414.6±35.8	42.1±6.8		33		
	BrS_A266V+R1629X with I_{K1} injection ER 1 Hz	-85.0±0.3	116.1±2.7	31.1±2.6	173.8±20.4		249.9±17.7	267.4±17.9	36.4±6.9		11		
	BrS_A266V+R1629X with I_{K1} injection ER 0.5 Hz	-84.5±0.4	112.8±2.9	28.3±2.8	148.3±37.0		214.9±39.7	231.8±40.2	36.5±7.4		11		
	BrS_A266V+R1629X with I_{K1} injection ER 0.11 Hz	-81.6±0.9	103.5±4.1	21.9±3.6	31.8±8.7		65.7±10.4	87.6±11.4	35.3±7.0		11		
	CON2 with I_{K1} injection patched at 24°C 1 Hz			40±2				510±100	110±30		9		
	CON2 with I_{K1} injection patched at 24°C 0.1 Hz			42±2				600±190	130±30		9		
	BrS2 p.T1620M with I_{K1} injection patched at 24°C 1 Hz			43**±1**				370±10	190±20		11		
	BrS2 p.T1620M with I_{K1} injection patched at 24°C 0.1 Hz			44±1				390±20	205±20		11		
	BrS2 p.T1620M with I_{K1} injection patched at 34°C 1 Hz			55±4				505±20	380±10		6		
	BrS2 p.T1620M with I_{K1} injection patched at 34°C 0.1 Hz			56±5				610±90	395±5		6		

	BrS_A266V+R1629X with I_{K1} injection 0.1 Hz	-82.9±0.9	107.6±4.6	24.7±4.0	53.1±21.3		116.2±31.5	127.5±29.7	60.7±12.4		9		
	BrS_A266V+R1629X with I_{K1} injection 0.1 Hz +0.5 mM 4-AP	-83.6±0.6	114.7±1.4	31.1±1.2	405.9±28.18		551.5±23.1	579.2±23.7	52.0±10.0		9		
	BrS_A266V+R1629X with I_{K1} injection 1 Hz	-84.8±0.4	115.6±2.2	30.8±1.9	199.9±18.7		288.9±19.4	308.5±19.7	31.4±7.7		9		
	BrS_A266V+R1629X with I_{K1} injection 1 Hz +Flecainide	-85.0±0.3	109.6±2.1	24.6±1.8	104.0±10.5		157.4±12.3	170.9±12.4	12.6±2.7		9		
56	WT (average from both controls)	-70±3	108±4				1802±494		23±7		16	Dissociated cells at 37 degrees patched 20-46 days after the start of differentiation	0 to -100 pA
	WT with I_{K1} injection (average from both controls)	-82±1	135±4				1250±280		121±13		21		
	WT1								140±12		12		
	WT2								98±10		9		
	MUT1								22±4		32		
	MUT2								41±9		29		
	MUT (average from both mutants)	-65±2	104±2				2274±314		7±1		41		
	MUT with I_{K1} injection (average from both mutants)	-82±1	120±2				1350±150		31±5		61		
88	hiPSC-CM control	-72.4±0.9	106±3.2		50.7±6.2		89.8±7.9	173.5±12.2	115.7±18.4		16	Single CMs, after day 50 of differentiation; data from 4 independent differentiations per line. Recordings on quiescent cells that contracted upon field stimulation; recordings performed at 36°C; APs elicited at 1,2 or 3 Hz by 3ms, 1.2x threshold current pulses	
	hiPSC-CM het	-71.3±1.3	103.1±3.2		58.7±5.9		109±10.1	217.2±14.9	57.6±14		13		

												through the patch pipette; average from 10 consecutive AP waveforms	
86	Control	-65±4	99.3±3.9	34.4±2.5			247.3±30.5	416.7±24	15.8±3.8		10	iPSC-CMs after day 30	
	LQTS/BrS	-60±2.8	97.4±4	37.4±1.8			326.1±74.5	563.7±57.1	21.8±10.8		7		
	corrected	-53.8±1.9	93.1±2.9	39.3±1.2			250.9±40	418±29.6	8.6±1.6		10		
69	Control D1	-82±1	138±4				102±13	290±5	42±3		22	Dissociated cells at D40-60 of differentiation, Patching at RT	Yes
	Control D2	-82±1	140±2				142±18	295±5	39±2		21		
	Control D3	-82±1	130±2				98±13	230±5	38±3		22		
	BrS SCN10A	-81±1	125±2				115±15	285±5	23±2		19		
	Control D1 +3 µM ajmaline	-81±1	140±2				90±12	220±5	43±3		11		
	Control D1 +10 µM ajmaline	-81±1	138±2				80±12	219±5	41±3		11		
	Control D1 +30 µM ajmaline	-81±1	135±2				79±12	219±5	36±3		11		
	BrS SCN10A +3 µM ajmaline	-80±1	120±2				116±15	253±7	25±2		15		
	BrS SCN10A +10 µM ajmaline	-81±1	110±2				125±15	280±7	21±2		15		
	BrS SCN10A +30 µM ajmaline	-81±1	108±2				140±15	305±7	20±1		15		
70	Control D1	-82±1	138±4				102±13	290±5	42±3		22	Dissociated cells at D40-60 of differentiation, Patching at RT	Yes
	Control D2	-82±1	140±2				142±18	295±5	39±2		19		
	Control D3	-82±1	130±2				100±13	285±5	38±3		30		
	BrS	-82±1	103±2				150±20	320±10	28±3		17		
71	Ctl1					150±5	200±10	210±90	14±7	500±100	6	Patched on D31-35 with amphotericin-B on single cells at 37°C; cycle length 700ms	
	BrS1					210±100	290±120	400±200	9±7	1100±1900	16		
	Rad WT					250±60	390±200	400±300	16±29	600±600	7		
	Rad R211H ins					500±110	580±120	590±110	10±3	1100±800	7		
87	iPS-HS1M	-58±46	90±35					183.0±17.9	17±10		21	APs recorded at D42-56 of differentiation at 22±2°C	
	iBR1-P5M-L1	-50±10	88±37					125.5±12.4	17±10		17		
55	iCtrl 1	-80	115±2		65±6		120±8	155±8	225±13		30		

	iCtrl 2	-80	110±2		85±5		155±8	190±8	200±13		30	iPSC-CMs after day 19 at 36°C; APs elicited at 0.5-3 Hz by 3-ms, ~1.2x threshold current pulses; average from 10 consecutive APs	Constant 2pA/pF
	iBrS 1	-80	118±2		86±5		150±8	175±8	180±20		28		
	iBrS 2	-80	115±2		55±4		100±5	130±5	195±13		30		
	iBrS 3	-78	118±4		80±5		149±8	177±8	260±15		19		
	iSCN5A	-78	126±2		110±5		180±5	240±20	126±25		22		

Supplementary Table 3: I_{Na} properties from published BrS iPSC-CM models. κ – slope factor of activation/inactivation curve; $V_{1/2}$- mid-point of activation/inactivation; τ_f- fast kinetics; τ_s- slow kinetics; I_{NaL}- late/persistent sodium current density; RT – room temperature

Reference	Cell line	Sodium current properties																Protocol
		I_{Na} peak density		Activation			Inactivation					Recovery			I_{NaL}		Cell capacitance		
		pA/pF	n	κ	$V_{1/2}$ [mV]	n	κ	$V_{1/2}$ [mV]	τ_f [ms]	τ_s [ms]	n	τ_f [ms]	τ_s [ms]	n	pA/pF	n	pF	n	
89	hiPSC-CM^{WT1}	-105± 15	11															Recordings at RT; cycle lenght 5s, -5mV hyperpolarizing step from -50 mV
	hiPSC-CM^{WT2}	-102± 13	21															
	hiPSC-CM^{R1638X}	-30± 2	11															
	hiPSC-CM^{W156X}	-32± 3	10															
68	CON1	-122.8± 31.3	10-25														22.2± 4	10-25	INa recordings at RT; cycle lenght 40ms; holding potential -80 mV
	CON2																	
	BrS1	-33.7± 6.3	10-25														27± 1.7	10-25	
	BrS2	-36.8± 8.6	10-25														24.9± 2.2	10-25	
	BrSp2-GE	-63.0±4.3	10-25														21.9± 2.6	10-25	
90	CON1	-245.8± 32.7	15	4.94± 0.4	-35.72± 0.46	15	7.55± 0.35	-70.26± 0.4			15	19.1± 0.05	327.4± 0.05	14					Patched at RT; cycle lenght 5s; holding potential -90 mV
	BrS_A266V+R1629X	-59.2± 8.8	21	4.99± 0.49	-40.89± 0.56	21	6.38± 0.19	-73.37± 0.22			15	23.3± 0.04	380± 0.05	14					
91	Beating bodies Control hiPSC-CM	-78.77± 5.16	3	5.57± 0.33	-44.15± 0.37	3	8.94± 1.54	-61.64± 0.77			3	5.85± 1.01	40.38± 4.95	3					Measurements at RT; cycle lenght 50 ms; holding potential -80 mV
	Beating bodies Patient hiPSC-CM	-42.93± 3.86	7	5.86± 0.28	-36.73± 0.32	7	8.7± 0.57	-70.15± 2.76			7	2.89± 0.35	34.45± 14.45	6					
	Monolayer Control hiPSC-CM	-45.62± 5.37	11	3.94± 0.14	-32.96± 0.79	11	6.7± 0.33	-48.8± 0.79			8	2.58± 0.31	46.17± 7.01	5					
	Monolayer Patient hiPSC-CM	-30.51± 3.09	13	5.58± 0.26	-25.44± 0.78	13	9.59± 0.24	-54.69± 1.21			10	1.68± 0.18	20.12± 6.76	5					

56	WT (average from both controls)	-279± 53	20		-58± 0.4	20		-77± 0.3			17	2.4± 0.2	62± 28	17					Measurements at RT; cycle lenght 3s; holding potential -120 mV
	MUT1	-52± 10	31		-31± 2	31		-85± 2			31								
	MUT2	-96± 15	26		-40± 3	26		-84± 2			25								
	MUT (average from both mutants)	-68± 6	57		-35± 0.5	57		-84± 0.1			57	2.7± 0.3	301± 46	57					
88	hiPSC-CM control	-264.4± 57	13												0.5± 0.1	9	36± 3.3	13	Measurements at RT; cycle lenght 5s; holding potential -90 mV
	hiPSC-CM het	-121.4± 23.8	13												1.8± 0.2	9	31.7± 3.2	13	
86	Control	-200± 10	22												1.8± 0.2	22			Measurements at RT, at rate 0.33 Hz, holding potential of -100 mV
	LQTS/BrS	-180± 20	21												2.5± 0.25	21			
	Control in second experiment with corrected iPSC-CMs	-100± 25	6												0.3± 0.05	6			
	LQTS/BrS in second experiment with corrected iPSC-CMs	-80± 10	13												0.8± 0.1	7			
	corrected	-65± 10	7												0.1± 0.1	13			
69	Control D1	-116.9± 28.7	25												-2.6± 0.3 / inhibited -1.1± 0.1	10/ 10			Measurements at RT; cycle lenght 4s; holding potential -100 mV
	Control D2	-95.4 ± 41.6	12												-2.8± 0.4 / inhibited -0.7± 0.2	15/ 24			
	Control D3	-94.7 ± 28.3	11												-3.2± 0.8 / inhibited -1± 0.2	10/ 10			
	BrS SCN10A	-54.7± 9.4	37												-1.1± 0.1 / inhibited -0.6± 0.1	17/ 20			
70	Control D1	-116.9± 28.7	25		-53.2± 2.1	25		-73.3± 2.3			25	22± 8		25	-0.4± 0.06	12			Measurements at RT; cycle lenght 4s;

	Control D2	-95.4± 41.6	12		-43.3± 2.1	12		-80.6± 1.5			12	20± 2		12	-0.4± 0.08	16			holding potential -100 mV
	Control D3	-94.7± 28.3	11		-45.1± 4.1	11		-77.9± 2.6			11	22± 2		11	-0.3± 0.006	14			
	BrS	-19.3± 3.7	22		-37.8± 1.8	22		-88.1± 1.8			22	75± 15		22	-0.2± 0.03	14			
71	Ctl1	-58.8± 16.5	17	5.3± 0.2	-39.4± 1.3	17	5.3± 0.1	-83.4± 1.3	1.1± 0.1	2.9± 0.3	9	9.7± 0.6	109.1± 6.8	13	0± 1	7	45.7± 22.8	51	Measurements at RT; cycle lenght 50 ms; holding potential -120 mV
	BrS1	-36.8± 16.7	42	5.2± 0.2	-36.1± 0.7	42	4.5± 0.6	-82.9± 0.8	1.2± 0.1	3.5± 0.2	20	6.7± 0.4	93.6± 3.3	33	-3± 5	40	55.5± 25.5	68	
	Rad WT	-56.8± 35.0	18	7.4± 0.1	-34.8± 0.6	9	6.9± 0.1	-83.3± 1.2			9								
	Rad R211H ins	-30.3± 14.2	14	8.4± 0.1	-28.1± 0.4	10	5.7± 0.2	-82.7± 1.5			10								
92	H9 hESC-CMs	-100± 60	7																Measurements at RT; 200 ms pusles; holding potential -120 mV
	AC patient iPSC-CMs	-20± 50	8																
	AC patient iPSC-CMs + PKP2 WT	-40± 20	8																
	AC patient iPSC-CMs + PKP2-R635Q	-25± 15	10																
87	iPS-HS1M	-110± 18.7	15																INa recordings at RT; cycle lenght 3 s; holding potential -90 mV
	iPS-HS1M +100 µM ajmaline	-15.4± 4.4	15																
55	iCtrl 1	-93.6± 84.9	36	6.9± 0.1	-34.6± 0.5	26	6.48± 0.2	-78.9± 1.4			15	1.5± 0.1	5.2± 0.5	36					Measurements at RT; cycle lenght 5s; holding potential -100 mV
	iCtrl 2	-83± 83.3	31	7± 0.2	-33± 0.9	20	7.05± 0.3	-85± 1			11	1.5± 0.1	5.9± 0.7	33					
	iBrS 1	-81.7± 73.25	37	6.6± 0.1	-33.5± 0.5	25	6.6± 0.2	-83.6± 0.9			15	1.52± 0.1	6.4± 0.7	37					
	iBrS 2	-96.8± 70.5	28	7± 0.2	-34.7± 0.7	20	6.6± 0.2	-84.5± 1			10	1.55± 0.1	7.2± 1	31					
	iBrS 3	-151.7± 208.5	31	7.2± 0.2	-32.1± 0.6	29	6.83± 0.2	-80± 0.9			13	1.68± 0.2	7.3± 0.8	32					
	iSCN5A	-38.7± 55.03	25	6.7± 0.2	-31.8± 0.9	20	6.9± 0.2	-83.7± 0.8			10	2.55± 0.2	16.5± 2	25					

Supplementary Table 4: Calcium current (I_{CaL}) properties from published BrS iPSC-CMs. $V_{1/2}$ – mid-point of activation/inactivation; κ – slope factor of activation/inactivation curve; τ_f- fast kinetics; τ_s- slow kinetics; RT – room temperature.

Reference	Cell line	I_{CaL} properties									Protocol
		Peak		Activation			Inactivation				
		pA/pF	n	$V_{1/2}$ [mV]	κ	n	τ_f [ms]	τ_s [ms]	$V_{1/2}$ [mV]	n	
56	WT (average from both controls)	-5.8±0.5	19								Not specified
	MUT (average from both mutants)	-8±1	57								
69	Control D1	-9.9 ±1.7	18	-7 ±1.8					-36 ±2.1	18	measurements at RT
	Control D2	-10.1 ±2.8	11	-6.6 ±1.3					-36.2 ±2.2	11	
	Control D3	-6.5 ±1.3	11	-5.5 ±1.5					-34.6 ±1.3	11	
	BrS SCN10A	-3.4 ±1.5	12	12.3 ±4.1					-47.1 ±3.2	12	
70	D1	-8.0±1.4	20	-7±2		20		15±5	-35±2	20	Not specified
	D2	-8.4±1.9	18	-6.5±1.5		18		13±6	-35±3	18	
	D3	-6.5±1.3	11	-5,9±1,1		11		11±5	-33±2	11	
	BrS	-7.4±2.4	13	-5±5.2		12		33±8	-33±9	13	
71	Ctl1	-26.3±7.1	34	-18.6±0.6	5.5±0.1	34					Not specified
	BrS1	-18.9±6.0	24	-18.6±0.6	5.8±0.1	24					
	Rad WT	-6.1±1.7	6	-14.1±0.6	6.7±0.4	6					
	Rad R211H ins	-1.7±0.5	8	-11.2±0.6	6.2±0.3	8					
55	iCtrl 1	-52±3	21				4.0±0.2	18.2±0.8			measurements at 36°C; cycle lengths 2s
	iCtrl 2	-58±3	21				3.7±0.2	21.5±1.3			
	iBrS 2	-60±3	19				3.6±0.3	21.5±1.4			

Supplementary Table 5: Calcium transient properties from published BrS iPSC-CMs. CTD50/75 – calcium transient duration at 50/75% of depolarization.

Reference	Cell line	CT properties						Protocol
		CTD50 [ms]	CTD 75 [ms]	Beat interval [ms]	Rise rate [ms]	Amplitude [mV]	n	
68	CON1	471.3±12.6		108.3±20.4	92.0±6.6	6±0.3	10-25	CT measured on dissociated cells with 5 µM Fluo-4 AM and 0.02% Pluronic F-127 at 37°C.
	CON2							
	BrS1	1362.7±124.6		1894.7±436.5	10.3±0.9	2.6±0.2	10-25	
	BrS2	541.5±60.2		313.3±59.1	45.2±4.7	2.3±0.2	10-25	
	BrSp2-GE	589.3±27.4		190.7±24.6	72.7±5.0	4.7±0.1	10-25	
71	Ctl1		500±150				5	CT measured on single CMs at 37°C.
	BrS1		790±180				7	
	Rad WT		390±400				22	
	Rad R211H ins		590±400				27	

Supplementary Table 6: Field potential properties from published BrS iPSC-CMs. FPD – field potential duration; BPM – beats per minute.

Reference	Cell line	FPD						Protocol
		Baseline [ms]	100 nM ajmaline [ms]	1 µM ajmaline [ms]	10 µM ajmaline [ms]	100 µM ajmaline [ms]	Beating rate [BPM]	
86	Control	210±5					65±17	Not specified
	LQTS/BrS	350±50					59±6	
87	iPS-HS1M	322.3±19.1	326.0±19.3	334.4±19.0	379.8±29.4	462.1±47.4		Sampling frequency 10 kHz at 37°C. One minute baseline recordings were taken after minimum 15 min superfusion of basal media, and 1 min drug recordings were taken after 6 min superfusion.
	iBR1-P5M-L1	319.6±12.8	327.4±13.6	352.1±12.6	385.7±13.3	415.2±26.1		
	iBR1-P5M-L9	281.3±15.0	307.7±13.8	328.7±15.0	364.8±16.4	418.7±23.1		
	iBR1-P3M-N2 (PKP2-R101H)	395.5±10.9	439.3±7.9	448.0±13.9	505.0±21.8	596.6±19.5		
	iBR1-P6M-L1	385.4±32.9	410.1±32.8	428.7±29.7	484.0±30.1	553.0±27.0		

Supplementary Table 7: I_{to} properties from published BrS iPSC-CMs. $V_{1/2}$- mid-point of inactivation

Reference	Cell line	I_{to} properties					Protocol
		Peak		Inactivation			
		pA/pF	n	Time constant [ms]	$V_{1/2}$	n	
56	WT (average from both controls)	15±1	18				Not specified
	MUT (average from both mutants)	6±1	59				
70	Control D1	2.7±0.4					Not specified
	Control D2	1.9±0.6					
	BrS	1.7±0.4					
55	iCtrl 1	14.5±2	13	28.1±4.6		13	Measurements at 36 °C; cycle length 10s
	iCtrl 2	11.5±2	13	26±5		13	
	iBrS 2	14.5±2.5	13	27.1±3.3		13	

Supplementary Table 8: I_{Kr} properties from published BrS iPS-CMs.

Reference	Cell line	Peak I_{Kr}				Protocol
		pA/pF	n	pA/pF +100 µM ajmaline	n	
70	Control D1	2.5±0.25	19			Not specified
	Control D2	2.2±0.4	11			
	BrS	1.1±0.2	13			
87	iPS-HS1M	1.7±0.2	8	0.7±0.1	8	Not specified

Supplementary Table 9: I_{Ks} properties from published BrS iPSC-CMs.

Reference	Cell line	Peak I_{Ks}		Protocol
		pA/pF	n	
64	Control D1	1.2±0.2	21	Not specified
	Control D2	1.25±0.25	14	
	BrS SCN10A	0.3±0.2	7	
65	Control D1	0.7±0.2	16	Not specified
	Control D2	0.7±0.3	13	
	Control D3	N/A		
	BrS	0.1±0.09	10	