Supplemental Material

Portero *et al.*: Dysfunction of the voltage-gated K+ channel beta-2 subunit in a familial case of Brugada syndrome

Correspondence should be addressed to Richard Redon, Inserm UMR 1087 / CNRS UMR 6291, l'institut du thorax, IRS-UN, 8 Quai Moncousu, Nantes, France. E-mail: richard.redon@inserm.fr. Tel: +33 (0)2 28 08 01 41.

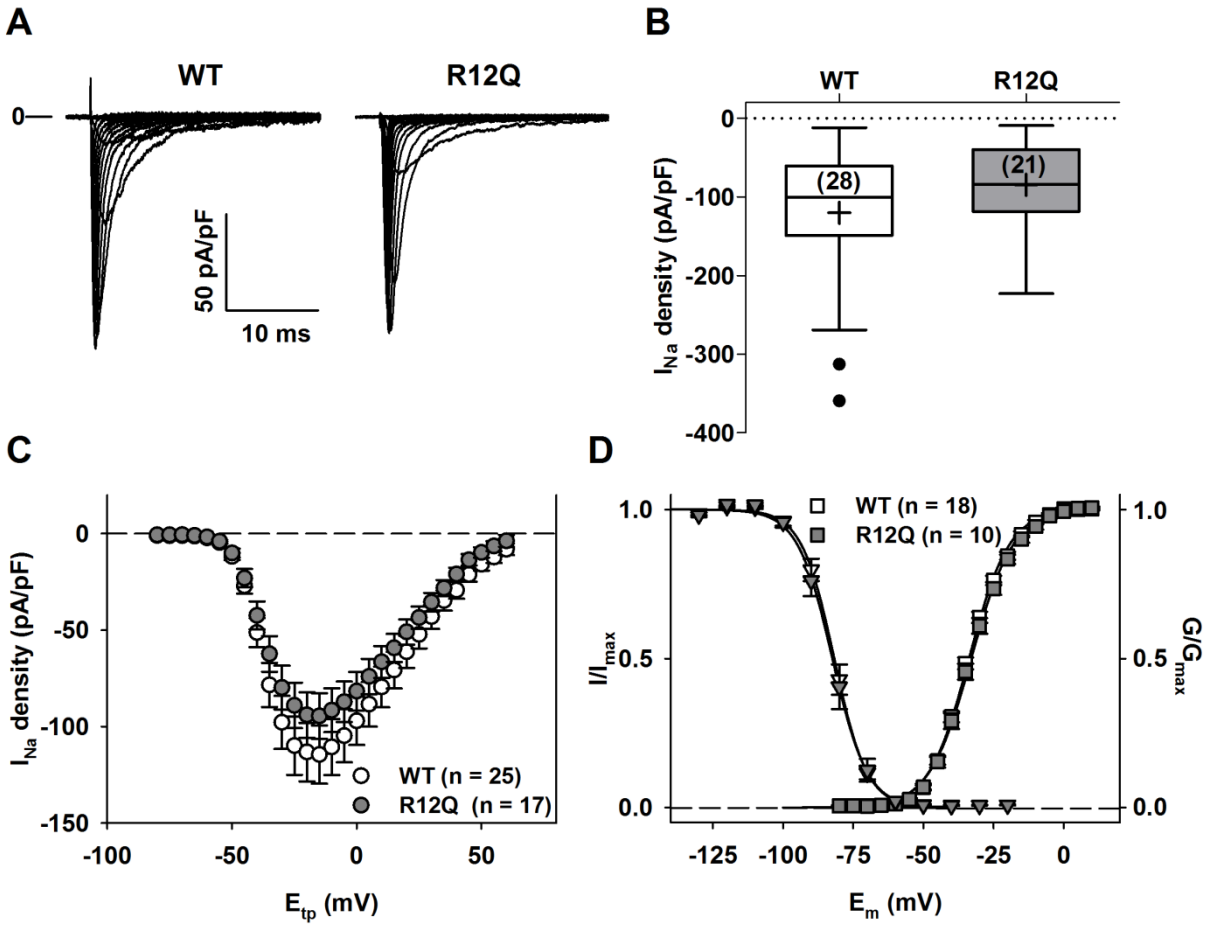

Figure S1

Absence of effect of R12Q Kvβ2 mutation on heterologously expressed Nav1.5-generated current. (A) Superimposed representative currents activated by a 50-ms pulse every 1 s to various potentials (from -80 mV to +60 mV, 5-mV increments; holding potential: -100 mV;

only one out of two pulses are shown for clarity) obtained from COS-7 cells expressing Nav1.5, Navβ1 and either wild-type (WT; left) or R12Q (right) Kvβ2. (B) Tukey plots of peak current (INa) densities recorded at -20 mV. Sample size is indicated in brackets, only outlier values are represented as symbols. (C) Mean INa density/voltage relationship of Nav1.5-generated currents in cells expressing Nav1.5, Navβ1 and either WT or R12Q Kvβ2. (D) Relative peak conductance (G/Gmax) versus test-pulse membrane potential (activation) and steady-state channel availability at -20 mV (I/Imax) versus prepulse potential (inactivation) plots for Nav1.5 channels. Inactivation voltage protocol: 500-ms polarization to variousprepulse potentials from –110 to –50 mV (10-mV increment) and test pulse at -20 mV for 20 ms (holding potential –100 mV; frequency 0.25 Hz). Curves are Boltzmann fits to the data.

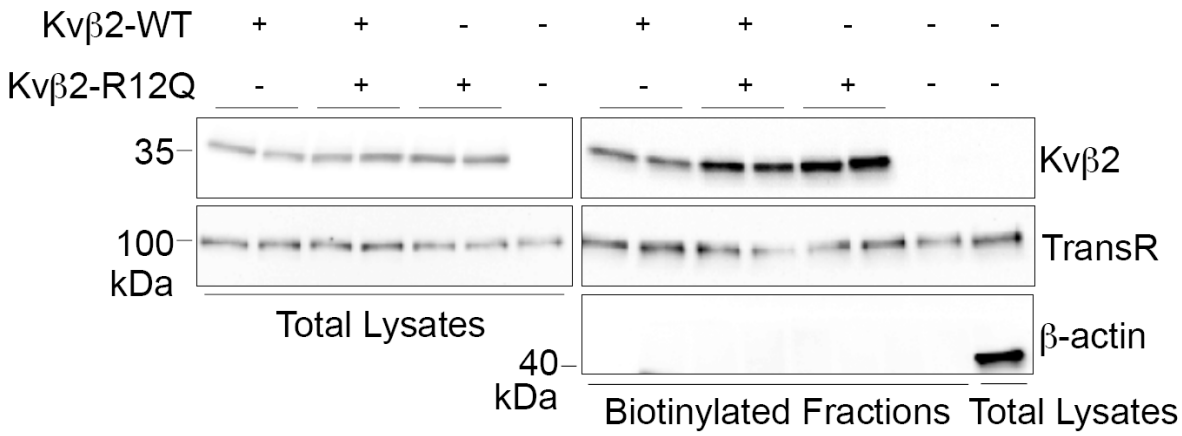

Figure S2

The submembrane expression of the heterologously expressed Kvβ2-R12Q protein is increased in the absence of Kv4.3. Forty-eight hours following transfection of COS-7 cells with cDNA constructs encoding Kvβ2-WT and/or Kvβ2-R12Q, cell lysates were prepared and used in cell surface biotinylation assays. Representative western blots of total (left) and biotinylated (right) Kvβ2 fractions from transfected COS-7 cells show that the R12Q mutation increases Kvβ2 submembrane protein expression in the absence of Kv4.3 and whether expressed alone or coexpressed with Kvβ2-WT. Western blot analyses of the transferrin receptor (TransR) and β-actin confirmed equal protein loading and absence of contamination of the biotinylated fractions by cytoplasmic proteins, respectively.

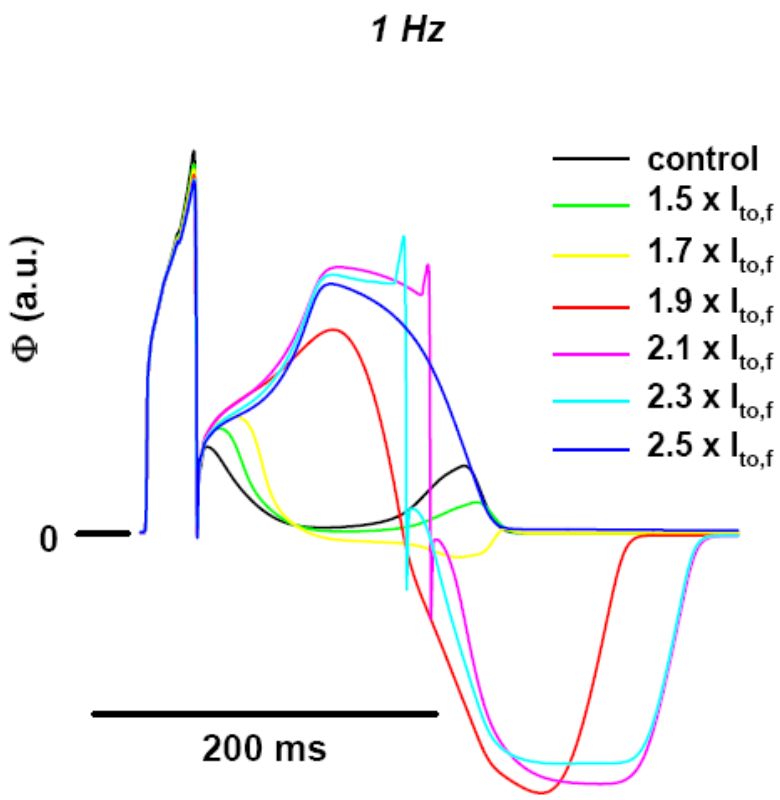

Figure S3

Modeling the effect of the R12Q variant at the heterozygous state. Right ventricular outflow tract wedge pseudo-ECGs, when the Kv4.3 current density is increased by a factor varying from the minimal value of 1.5, when considering only the lower limit for the heterozygous R12Q condition, to the averaged value of 2.5 (see figure 5A, a.u.: arbitrary units).

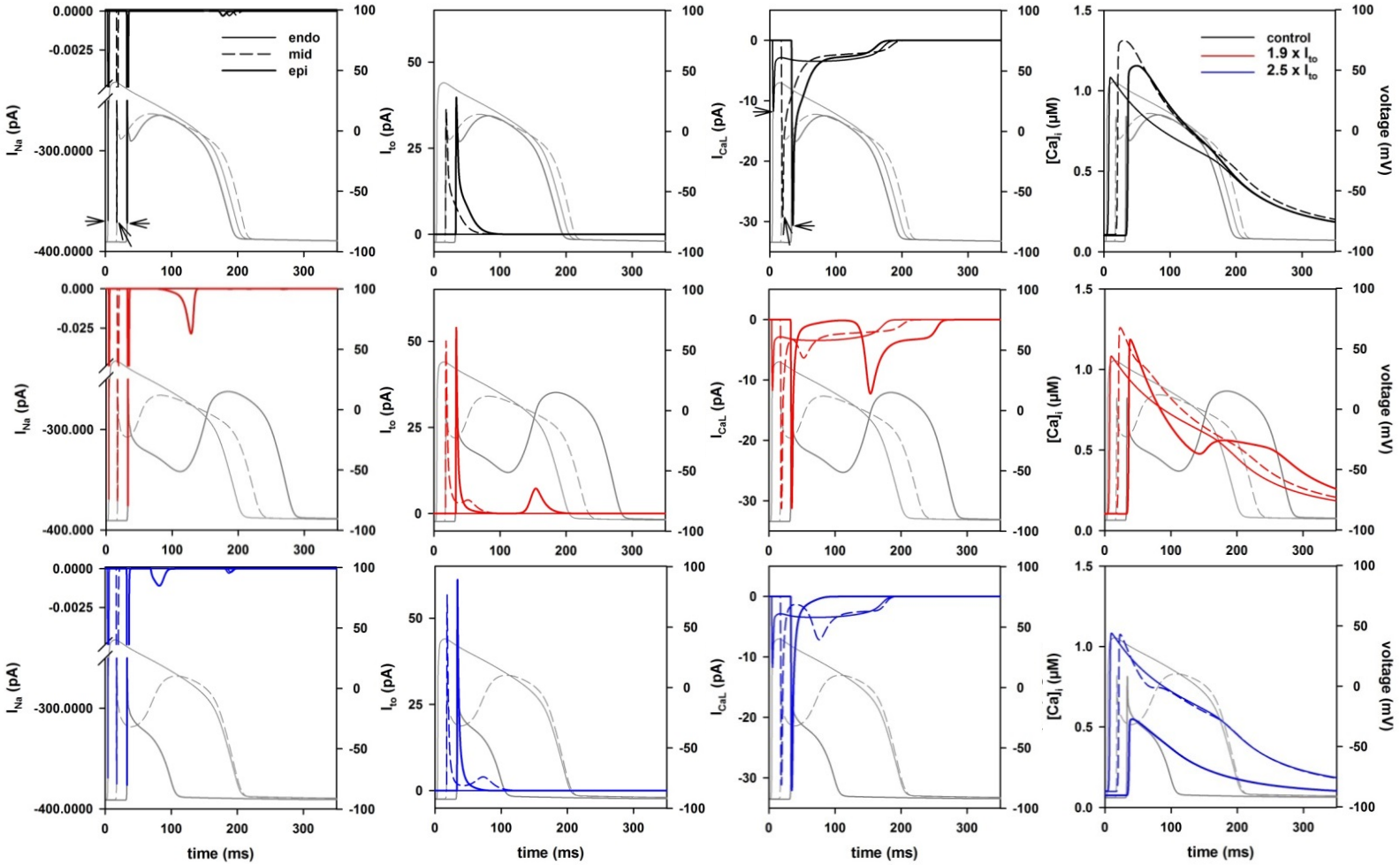

Figure S4

Ion currents and Ca2+ transients underlying the different APs (grey) in control condition (black) and in presence of a 1.9-fold (red) or a 2.5-fold (blue) increase in Ito,f. See multiple scales for INa graphs. Arrows indicate the peak currents in control condition.

Table S1. Clinical characteristics of the Kvβ2-R12Q family

		II.3 (proband)	II.1	II.4	I.2	I.1	II.2
Age at diagnosis		39	32	34	59	70	35
Holter monitoring		Polymorphic VT	Normal	Normal	Normal	Normal	Normal
Echocardiography		Normal	Normal	Normal	Normal	LVEF 45%, LVTDD 67 mm	Normal
Baseline ECG	HR (bpm)	86	78	63	75	85	63
	PR (ms)	173	167	190	184	165	165
	QRS (ms)	105	90	108	110	148	78
	QT (ms)	367	361	382	372	381	385
	QTc (ms)	439	412	391	416	453	395
	J V1 (mm)	2.5	0	0.5			
	J V2 (mm)	5	1	0			
	J V3 (mm)	2	0	0			
	max TPE in precordial leads (ms)	75	71	78			
Electrophysiological study	AH interval (ms)	96	105	119			
	HV interval (ms)	57	54	56			
	Programmed ventricular stimulation	FV	Normal	Normal			
	Ventricular refractory period (ms)	210	<200	<200			

Table S2. Rare variants co-segregating with the Brugada ECG phenotype in a familial case of Brugada syndrome

CHR	POS (hg19)	REF	ALT	Segregation	Gene	GERP	dbSNP	Consequence	HGVSc; HGVSp	SIFT; PolyPhen	I:1	I:2	II:1 (aff)	II:2	II:3 (aff)	II:4 (aff)
1	6100663	G	A	Segregation (inherited from father I:1)	*KCNAB2*	4.99	rs758297152	missense_variant	NM_003636.3:c.35G>A; NP_003627.1:p.Arg12Gln	tolerated_low_confidence(0.06); probably_damaging(0.953)	+	-	+	-	+	+
3	49159478	G	T	Segregation (inherited from father I:1)	*LAMB2*	5.55	rs777742373	missense_variant	NM_002292.3:c.4822C>A; NP_002283.3:p.Gln1608Lys	tolerated(1); benign(0.003)	+	-	+	-	+	+
5	125801239	T	C	Segregation (inherited from father I:1)	*GRAMD3*	4.72	rs200501308	splice_donor_variant	NM_001146319.1:c.248+2T>C	-	+	-	+	-	+	+
5	140078090	C	T	Segregation (inherited from father I:1)	*HARS2*	4.57	rs752851001	missense_variant	NM_001278731.1:c.1399C>T; NP_001265660.1:p.Arg467Trp	deleterious(0.01); probably_damaging(0.991)	+	-	+	-	+	+
9	113013717	G	A	Segregation (inherited from father I:1)	*TXN*	-6.21	rs146018216	missense_variant	NM_001244938.1:c.50C>T; NP_001231867.1:p.Ala17Val	tolerated(0.11); benign(0.001)	+	-	+	-	+	+
9	123636952	T	C	Segregation (inherited from father I:1)	*PHF19*	3.5	rs146513217	missense_variant	NM_001009936.1:c.68A>G; NP_001009936.1:p.Lys23Arg	tolerated_low_confidence(0.11); benign(0.008)	+	-	+	-	+	+

Table S3. *In silico* ranking of candidate genes

Gene	*Endeavour*			*ToppGene*		
	Rank	*Score*	*Rank ratio*	*Rank*	*Average Score*	*Overall pValue*
KCNAB2	**1**	**0.192**	**0.167**	**1**	**0.763**	**2.42×10^{-4}**
LAMB2	2	0.210	0.333	2	0.385	0.006
PHF19	3	0.606	0.500	5	0.455	0.262
TXN	4	0.616	0.667	3	0.332	0.091
GRAMD3	5	0.742	0.833	6	0.107	0.850
HARS2	6	0.981	1.00	4	0.040	0.242

Table S4. Absence of effects of R12Q mutation of Kvβ2 on sodium channel biophysical parameters.

	cell capacitance	current density	activation		inactivation		recovery from inactivation		
Nav1.5 + Kvβ2	**(pF)**	**at –20 mV (pA/pF)**	**$V_{1/2}$ (mV)**	**K (mV)**	**$V_{1/2}$ (mV)**	**K (mV)**	**A_{fast} (%)**	**τ_{fast} (ms)**	**τ_{slow} (ms)**
WT	20±1 (28)	–120.4±16.9 (28)	–33.7±0.6 (28)	6.9±0.2 (28)	–81.9±1.2 (18)	–5.0±0.1 (18)	79.2±3.1 (19)	32.7±2.9 (19)	597.5±65.3 (19)
R12Q	24±2 (21)	–84.8±11.5 (21)	–32.9±0.8 (19)	7.2±0.2 (19)	–82.6±1.8 (10)	–5.2±0.3 (10)	87.9±2.1 (9)	36.9±4.5 (9)	614.2±110.2 (9)

(n): number of cells; $V_{1/2}$ and K; voltage for half-activation or -inactivation of the Na^+ current and slope; A_{fast} and τ_{fast}: coefficient and time constant of the fast component of reactivation kinetics, τ_{slow}: time constant of the slow component. Voltage protocol of the recovery from inactivation studies: pre-pulse to -20 mV for 100 ms, repolarization to holding potential at -100 mV for various delays from 1 ms to 1000 ms with increasing increments followed by a 20-ms test pulse to -20 mV (frequency: 1/3 Hz).