

## **SUPPLEMENTAL MATERIAL**

**Table S1. *KCNQ1* and *KCNH2* LP/P Variants**

<b><i>KCNQ1</i> Variant</b>	<b>N (%)</b>
Total	164 (100)
c.403delG	1 (0.6)
Ala178Thr	1 (0.6)
Ala341Glu	9 (5.5)
Ala344Ala	1 (0.6)
Ala344Val	1 (0.6)
Arg174Leu	1 (0.6)
Arg190Gln	2 (1.2)
Arg195Pro	1 (0.6)
Arg231Cys	1 (0.6)
Arg243Cys	1 (0.6)
Arg259Cys	7 (4.3)
Arg366Trp	3 (1.8)
Arg518*	6 (3.7)
Arg555Gly	1 (0.6)
Arg555His	5 (3.0)
Arg591Cys	1 (0.6)
Arg591His	14 (8.5)
Arg594Gln	2 (1.2)
c.387-5T>A	3 (1.8)
c.477+1G>A	1 (0.6)
c.477+5 G>A	4 (2.4)
Gln505Ter	1 (0.6)
Gln530Ter	3 (1.8)
Glu284Lys	2 (1.2)
Glu284Ter	1 (0.6)
Glu449Argfs*14	1 (0.6)
Gly168Arg	4 (7.8)
Gly179Ser	1 (0.6)
Gly269Ser	2 (1.2)
Gly325Arg	4 (2.4)
Gly325Glu	2 (1.2)
Gly589Asp	3 (1.8)
Ile567Thr	1 (0.6)
Leu203Pro	4 (2.4)
Leu266Pro	10 (6.1)
Leu342Phe	1 (0.6)
Lys13Ter	2 (1.2)
Lys362Arg	5 (3.0)
Met159Ile	1 (0.6)
Pro343Leu	1 (0.6)
Ser225Leu	1 (0.6)

Ser277Pro	1 (0.6)
Ser546Leu	2 (1.2)
Ser566Phe	3 (1.8)
Trp305Leu	3 (1.8)
Tyr111Cys	2 (1.2)
Tyr125Asn	2 (1.2)
Tyr315Cys	1 (0.6)
Val205Met	31 (18.9)
Val254Met	2 (1.2)
<b>KCNH2 Variant</b>	<b>N (%)</b>
Total	51 (100)
Ala614Val	1 (2.0)
Arg100Trp	1 (2.0)
Arg176Trp	3 (5.9)
Arg366*	1 (2.0)
Arg534Cys	1 (2.0)
Arg582Cys	1 (2.0)
Arg784Trp	1 (2.0)
Arg823Trp	1 (2.0)
Asn633Ser	1 (2.0)
Asp259Argfs*73	1 (2.0)
Asp501Gly	1 (2.0)
c.234_241delTGCCGCGC	2 (3.9)
c.331_337dupGATGTGG	1 (2.0)
c.744dupC	1 (2.0)
c.925delC	2 (3.9)
Cys984Ter	2 (3.9)
Gln376Gln	1 (2.0)
Glu929GlyfsTer11	5 (9.8)
Gly1036Alafs*21	4 (7.8)
Gly1036Alafs*22	1 (2.0)
Gly47Asp	1 (2.0)
Gly584Ser	5 (9.8)
Gly601Ser	1 (2.0)
His562Arg	1 (2.0)
Leu559Phe	1 (2.0)
Ser182*	1 (2.0)
Ser818Leu	1 (2.0)
Thr613Met	1 (2.0)
Tyr43Cys	1 (2.0)
Val822Leu	1 (2.0)

LP indicates Likely-Pathogenic; P, pathogenic

**Table S2. QT Correction for Variant Carriers Using Other Formulas**

	<b>Framingham</b>	<b>Fredericia</b>	<b>Hodges</b>
Resting QTc (mean, SD)	433 (51)	447 (41)	446 (41)
Standing QTc (mean, SD)	418 (52)	451 (43)	449 (41)
Peak Exercise QTc (mean, SD)	308 (55)	411 (60)	460 (43)
1-Minute Recovery QTc (mean, SD)	348 (59)	426 (57)	443 (44)
4-Minute Recovery QTc (mean, SD)	410 (63)	458 (57)	457 (47)

QTc indicates corrected QT interval

QTc is expressed in ms (milliseconds)

**Table S3. Normative Treadmill Testing QTc in Non-Carriers**

	<b>95<sup>th</sup> percentile</b>		<b>98<sup>th</sup> percentile</b>	
	Males	Females	Males	Females
Supine QTc	455	456	465	470
Standing QTc	479	495	503	519
Peak Exercise QTc	519	519	557	551
1-Minute Recovery QTc	473	495	493	564
4-Minute Recovery QTc	469	480	531	495

QTc indicates corrected QT interval  
QTc is expressed in ms (milliseconds)

**Table S4. QTc and HR in Pre-Pubescent vs. Post-Pubescent LQTS Patients**

	<b>Pre-Pubescent LQTS (N=44)</b>	<b>Post-Pubescent LQTS (N=164)</b>	<b>p-value*</b>
Male (n, %)	22 (50)	65 (40)	
Female (n, %)	22 (50)	99 (60)	
Resting QTc (mean, SD)	447 (35)	456 (44)	0.14
Standing QTc (mean, SD)	463 (44)	470 (46)	0.42
Peak Exercise QTc (mean, SD)	476 (74)	474 (63)	0.71
1-Min Recovery QTc (mean, SD)	456 (47)	476 (62)	0.08
4- Min Recovery QTc (mean, SD)	464 (25)	491 (60)	<0.001

QTc indicates corrected QT interval; HR, heart rate

QTc is expressed in ms (milliseconds); HR is expressed in bpm (beats per minute)

\* Adjusted for familial relatedness and multiple comparisons

**Table S5. QT and HR in Symptomatic vs. Asymptomatic Patients**

	<b>Symptomatic Patients (N=80)</b>	<b>Asymptomatic Patients (N=128)</b>	<b>p-value*</b>
Resting QTc	462 (52)	450 (33)	0.20
Resting HR	66 (12)	68 (14)	0.28
Standing QTc	472 (54)	467 (38)	0.64
Standing HR	73 (16)	79 (15)	0.05
Peak Exercise QTc	478 (89)	475 (46)	0.08
Peak Exercise HR	144 (27)	148 (25)	0.29
Min-1 Recovery QTc	477 (69)	469 (52)	0.80
Min-1 Recovery HR	113 (23)	116 (26)	0.46
Min-4 Recovery QTc	498 (64)	477 (50)	0.02
Min-4 Recovery HR	84 (14)	89 (21)	0.07

QTc indicates corrected QT interval; HR, heart rate

QTc is expressed in ms (milliseconds); HR is expressed in bpm (beats per minute)

\*Adjusted for familial relatedness and multiple comparisons

**Table S6. Predictive Utility of Exercise ECG Characteristics\***

	Males (N=168)						Females (N=208)						P-value
	AUC	Cutoff (ms)	Specificity	Sensitivity	PPV	NPV	AUC	Cutoff (ms)	Specificity	Sensitivity	PPV	NPV	
Resting	0.82	470	0.99	0.22	1	0.60	0.84	480	0.96	0.27	1	0.55	0.32
Standing	0.69	417	0.51	0.81	0.50	0.79	0.76	433	0.61	0.81	0.62	0.81	0.17
Peak Exercise	0.66	416	0.37	0.81	0.47	0.74	0.78	431	0.64	0.81	0.64	0.81	0.06
1-Min Recovery	0.77	410	0.56	0.81	0.57	0.81	0.85	427	0.79	0.81	0.76	0.84	0.002
4-Min Recovery	0.82	442	0.76	0.81	0.67	0.86	0.9	452	0.87	0.81	0.79	0.84	0.22

AUC indicates area under the curve; QTc, corrected QT interval; PPV, positive predictive value; NPV, negative predictive value; p-values compare males vs. females

\*Modelling adjusted for age and familial relatedness, and p-values corrected for multiple comparisons



**Table S7. Cut off Values for 4-Minute Recovery QTc at Higher Specificities**

<b>Specificity</b>	<b>Sex</b>	<b>Cutoff (ms)</b>	<b>Sensitivity</b>	<b>PPV</b>	<b>NPV</b>
0.80	Males	445	0.86	0.73	0.85
	Females	450	0.82	0.79	0.84
0.90	Males	460	0.67	0.80	0.76
	Females	463	0.60	0.85	0.76
0.95	Males	475	0.41	0.87	0.72
	Females	482	0.36	0.95	0.65
0.99	Males	586	0.02	1	0.61
	Females	563	0.02	1	0.55

PPV indicates positive predictive value; NPV, negative predictive value; PPV, positive predictive value; NPV, negative predictive value; QTc, corrected QT interval

**Table S8. Applied Cut-Off Values from Original Algorithm\***

		<b>Cut-Off</b>	<b>AUC</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>PPV</b>	<b>NPV</b>	<b>P-value</b>
<b>Step 1</b> (Resting QTc)	Males	470	0.82	0.22	0.99	1	0.60	-
	Females	480	0.84	0.27	0.96	1	0.55	-
<b>Step 2</b> (4-Min Recovery QTc)	Males	445	0.81	0.77	0.80	0.71	0.85	0.45
	Females		0.87	0.86	0.76	0.74	0.88	0.77
<b>Step 3</b> (1-Min Recovery QTc)	Males	460	0.70	0.61	0.63	0.82	0.36	0.34
	Females		0.85	0.76	0.97	0.98	0.46	1

PPV indicates positive predictive value; NPV, negative predictive value; AUC, area under the curve; QTc, corrected QT interval; p-value compares the predictive value of the original cut off values vs. newly proposed sex-stratified cut off values

\*Modelling adjusted for age and familial relatedness, and p-values corrected for multiple comparisons

**Table S9. p.A341-neighbouring vs. other variant-sites in LQTS1**

	<b>p.A341-Neighbouring</b>	<b>Non-p.A341-Neighbouring</b>	<b>P-value*</b>
N	11	152	
Males	5	61	
Females	6	91	
Resting QTc (mean, SD)	448 (34)	453 (43)	0.57
Standing QTc (mean, SD)	474 (42)	466 (47)	0.57
Peak Exercise QTc (mean, SD)	497 (37)	483 (66)	0.09
1-Min Recovery QTc (mean, SD)	488 (37)	482 (61)	0.60
4-Min Recovery QTc (mean, SD)	461 (17)	486 (61)	0.10

QTc indicates corrected QT interval; QTc is expressed in ms (milliseconds)

\*Adjusted for familial relatedness and corrected for multiple comparisons

**Table S10. Pore vs. non-pore variant site in LQTS2**

	<b>Pore</b>	<b>Non-pore</b>	<b>P-value*</b>
N	11	34	
Males	8	13	
Females	3	21	
Resting QTc (mean, SD)	460 (32)	458 (45)	0.65
Standing QTc (mean, SD)	473 (32)	479 (39)	0.67
Peak Exercise QTc (mean, SD)	442 (63)	448 (53)	0.67
1-Min Recovery QTc (mean, SD)	442 (38)	432 (35)	0.21
4-Min Recovery QTc (mean, SD)	491 (32)	491 (41)	0.26

QTc indicates corrected QT interval; QTc is expressed in ms (milliseconds)

\*Adjusted for familial relatedness and corrected for multiple comparisons

**Table S11. Pre- and Post-Beta Blockade QTc and HR at Various Stages of Treadmill Testing in Carriers**

	Carriers		P-value*
	Pre-BB (N=122)	Post-BB (N=129)	
Resting QTc (mean, SD)	461 (45)	449 (37)	0.001
Resting HR (mean, SD)	71 (13)	62 (11)	<0.001
Standing QTc (mean, SD)	478 (52)	462 (38)	0.01
Standing HR (mean, SD)	82 (16)	72 (14)	<0.001
Peak Exercise QTc (mean, SD)	480 (76)	468 (47)	0.20
Peak Exercise HR (mean, SD)	155 (25)	138 (23)	<0.001
Min 1 Recovery QTc (mean, SD)	474 (65)	471 (49)	0.18
Min 1 Recovery HR (mean, SD)	123 (24)	105 (22)	<0.001
Min 4 Recovery QTc (mean, SD)	488 (65)	484 (42)	0.10
Min 4 Recovery HR (mean, SD)	92 (20)	80 (14)	<0.001

BB indicates beta blockade, QTc, corrected QT interval, HR, heart rate

QTc is expressed in ms (milliseconds); HR is expressed in bpm (beats per minute)

\*Corrected for multiple comparisons

**Table S12. Utility of Step 2 and 3 in Male Patients with a Resting QTc < 470ms and in Female Patients with a Resting QTc < 480ms\***

	Males (N=68)					Females (N=89)				
	AUC	Specificity	Sensitivity	PPV	NPV	AUC	Specificity	Sensitivity	PPV	NPV
4-Min Recovery QTc	0.82	0.75	0.80	0.66	0.85	0.87	0.83	0.80	0.79	0.84
1-Min Recovery QTc	0.69	0.38	0.71	0.84	0.21	0.87	0.83	0.75	0.95	0.42

AUC indicates area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; QTc, corrected QT

\*Adjusted for age and familial relatedness, and p-values corrected for multiple comparisons

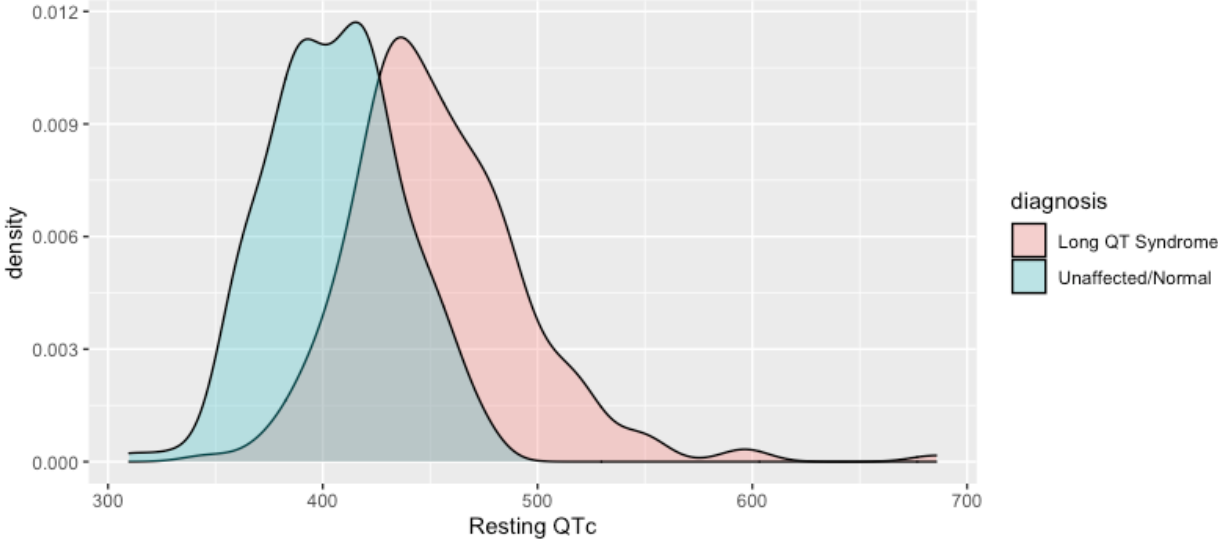
**Table S13. Performance of the Algorithm in Beta-Blocker Naïve Patients\***

	Males (N=56)					Females (N=66)				
	AUC	Specificity	Sensitivity	PPV	NPV	AUC	Specificity	Sensitivity	PPV	NPV
Resting QTc	0.84	0.99	0.25	1	0.71	0.89	0.99	0.30	1	0.71
4-Min Recovery QTc	0.85	0.74	0.82	0.56	0.91	0.85	0.85	0.74	0.67	0.89
1-Min Recovery QTc	0.74	0.74	0.71	0.90	0.44	0.68	0.69	0.65	0.93	0.23

AUC indicates area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; QTc, corrected QT

\* Adjusted for age and familial relatedness, and p-values corrected for multiple comparisons

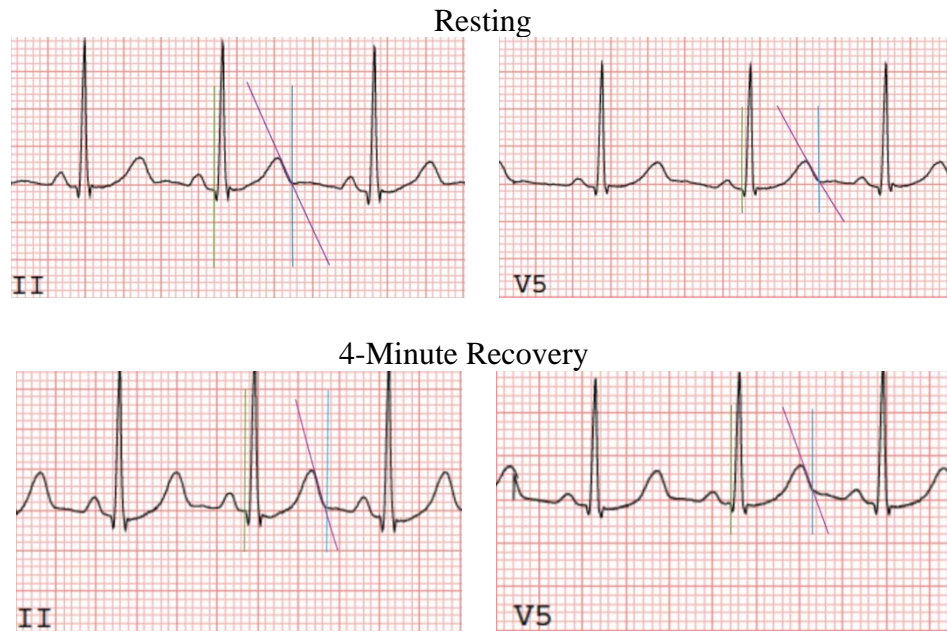
**Figure S1. Density Plot of Resting QTc**



QTc indicates corrected QT interval



**Figure S2. Clinical Application of the Algorithm**



Resting and 4-Minute Recovery strips (Lead II & V5) in an asymptomatic 21-year-old woman with a family history of LQTS who presented with a borderline QT interval of 461ms. The treadmill test showed prolongation of the QT in the 4-minute recovery period at 478ms.