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CLINICAL RESEARCH

Effects of cohort, genotype, variant, and maternal β-blocker treatment on foetal heart rate predictors of inherited long QT syndrome

Alexander M. Kaizer [1†], Annika Winbo [2,3†], Sally-Ann B. Clur [4,5], Susan P. Etheridge [6], Michael J. Ackerman [7,8,9,10], Hitoshi Horigome [11], Ulrike Herberg [12,13], Federica Dagradi [14], Carla Spazzolini [14], Stacy A.S. Killen [15], Annette Wacker-Gussmann [16], Arthur A.M. Wilde [5,17,18], Elena Sinkovskaya[19], Alfred Abuhamad[19], Margherita Torchio[14], Chai-Ann Ng [20,21], Annika Rydberg [2,5], Peter J. Schwartz [14]*‡, and Bettina F. Cuneo [22]*‡

[1]Biostatistics and Informatics, Colorado School of Public Health, University of Colorado-Anschutz Medical Campus, Aurora, CO, USA; [2]Department of Clinical Sciences, Pediatrics, Umeå University, Umea, Sweden; [3]Department of Physiology, University of Auckland, Auckland, New Zealand; [4]Department of Pediatric Cardiology, Emma Children's Hospital, Amsterdam University Medical Centers, Amsterdam, The Netherlands; [5]Department of Cardiology, University Medical Center, Amsterdam, The Netherlands; [6]Department of Pediatrics, Division of Cardiology, University of Utah School of Medicine, Salt Lake City, UT, USA; [7]Department of Cardiovascular Medicine, Division of Heart Rhythm Services, Mayo Clinic, Rochester, MN, USA; [8]Department of Pediatric and Adolescent Medicine, Division of Pediatric Cardiology, Mayo Clinic, Rochester, MN, USA; [9]Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA; [10]Windland Smith Rice Genetic Heart Rhythm Clinic and Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, MN, USA; [11]Department of Pediatrics, Section of Cardiology, Tsukuba University, Tsukuba, Japan; [12]Department of Pediatric Cardiology, RWTH University Hospital Aachen, Aachen, Germany; [13]Department of Pediatric Cardiology, University Hospital Bonn, Bonn, Germany; [14]Center for Cardiac Arrhythmias of Genetic Origin and Laboratory of Cardiovascular Genetics, IRCCS Istituto Auxologico Italiano, Via Pier Lombardo 22, 2015 Milan, Italy; [15]Department of Pediatrics, Division of Cardiology, Vanderbilt University Medical Center, Nashville, TN, USA; [16]Department of Congenital Heart Disease and Paediatric Cardiology, German Heart Center, Munich, Germany; [17]Department of Cardiology, Amsterdam UMC Location University of Amsterdam, Amsterdam, The Netherlands; [18]Department of Cardiology, Amseterdam University Medical Center, Amsterdam, The Netherlands; [19]Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Eastern Virginia Medical School, Norfolk, VA, USA; [20]Mark Cowley Lidwill Research Program in Cardiac Electrophysiology, Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia; [21]The School of Clinical Medicine, UNSW Sydney, Darlinghurst, New South Wales, Australia; and [22]Department of Pediatrics, Section of Cardiology, University of Denver School of Medicine, 13123 16th Ave, Box 100, Aurora, CO 80045, USA

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Aims

In long QT syndrome (LQTS), primary prevention improves outcome; thus, early identification is key. The most common LQTS phenotype is a foetal heart rate (FHR) < 3rd percentile for gestational age (GA) but the effects of cohort, genotype, variant, and maternal β-blocker therapy on FHR are unknown. We assessed the influence of these factors on FHR in pregnancies with familial LQTS and developed a FHR/GA threshold for LQTS.

Methods and results

In an international cohort of pregnancies in which one parent had LQTS, LQTS genotype, familial variant, and maternal β-blocker effects on FHR were assessed. We developed a testing algorithm for LQTS using FHR and GA as continuous predictors. Data included 1966 FHRs at 7–42 weeks' GA from 267 pregnancies/164 LQTS families [220 LQTS type 1 (LQT1), 35 LQTS type 2 (LQT2), and 12 LQTS type 3 (LQT3)]. The FHRs were significantly lower in LQT1 and LQT2 but not LQT3 or LQTS negative. The LQT1 variants with non-nonsense and severe function loss (current density or β-adrenergic response) had lower FHR. Maternal β-blockers potentiated bradycardia in LQT1 and LQT2 but did not affect FHR in LQTS negative. A FHR/GA threshold predicted LQT1 and LQT2 with 74.9% accuracy, 71% sensitivity, and 81% specificity.

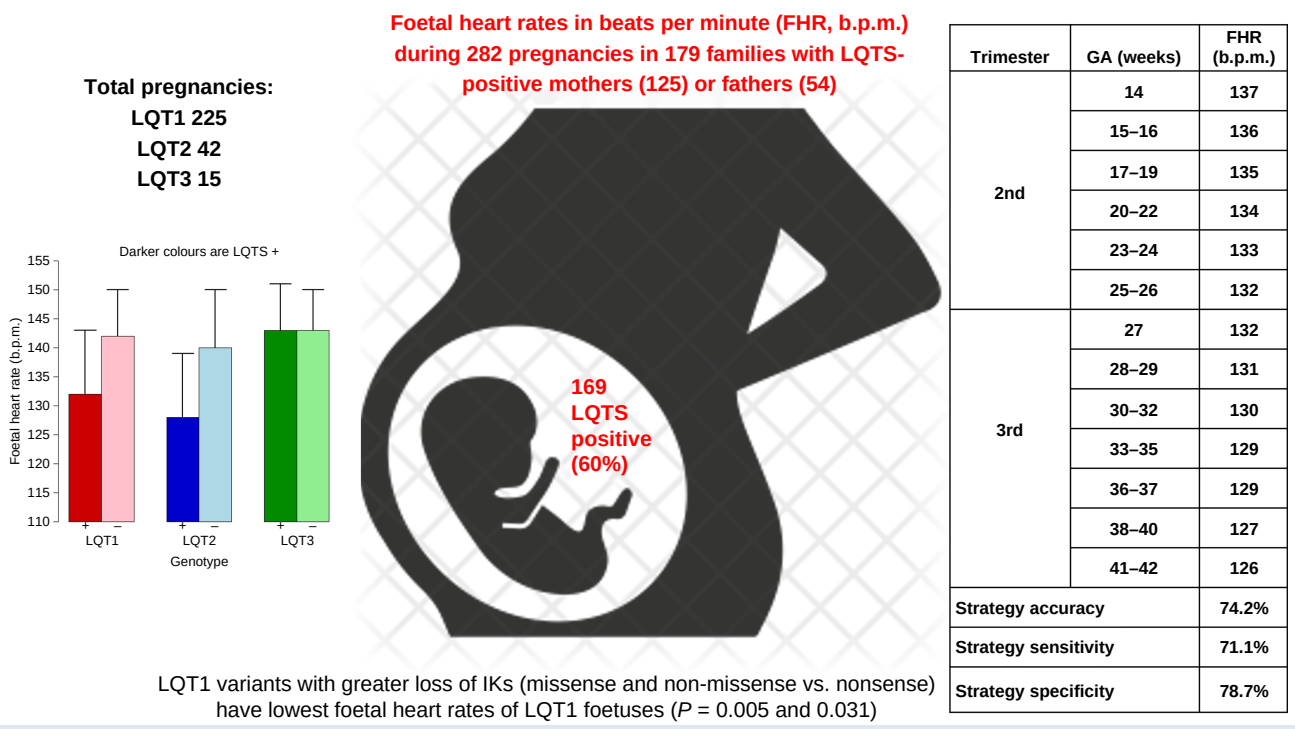
* Corresponding authors. Tel: +1 708 903 6318. *E-mail address*: Bettina.Cuneo@gmail.com (B.F.C.); Tel: +39 02 61911 1. *E-mail addresses*: p.schwartz@auxologico.it, peter.schwartz@unipv.it (P.J.S.)

† The first two authors contributed equally as first authors.

‡ The last two authors contributed equally as senior authors.

Conclusion Genotype, LQT1 variant, and maternal β-blocker therapy affect FHR. A predictive threshold of FHR/GA significantly improves the accuracy, sensitivity, and specificity for LQT1 and LQT2, above the infant's *a priori* 50% probability. We speculate this model may be useful in screening for LQTS in perinatal subjects without a known LQTS family history.

Graphical Abstract



Trimester	GA (weeks)	FHR (b.p.m.)
2nd	14	137
	15–16	136
	17–19	135
	20–22	134
	23–24	133
	25–26	132
3rd	27	132
	28–29	131
	30–32	130
	33–35	129
	36–37	129
	38–40	127
	41–42	126
Strategy accuracy		74.2%
Strategy sensitivity		71.1%
Strategy specificity		78.7%

Summary of findings from the manuscript.

Keywords Long QT syndrome • Channelopathy • Foetus • Foetal arrhythmia • Inherited arrhythmias • Bradycardia • Potassium currents • Stillbirth • Sudden death

What's new?

Novel findings from this study include the following:

- Positive long QT syndrome type 1 (LQT1) foetal heart rates (FHRs) did not differ between Swedish founder and consortium cohorts.
- Maternal β-blockers do not significantly decrease FHR in long QT syndrome (LQTS)-negative or LQT3 foetuses but potentiate the intrinsic bradycardia of LQT1 and LQT2 foetuses.
- Severe LQT1 variants have lower FHR than mild LQT1 variants.
- Our FHR/gestational age (GA) model predicted inherited LQT1 and LQT2 with 71% sensitivity and 81% specificity and should improve perinatal LQTS ascertainment and allow for earlier primary prevention of lethal cardiac events.
- The findings of this study have implications for the management of both the known LQTS pregnancy and for the evaluation of the foetus with no known family history but consistent FHRs meeting the criteria for LQTS.

Introduction

Because pharmacologic therapy in primary prevention is associated strongly with decreased morbidity and mortality, early identification and treatment of patients with long QT syndrome (LQTS) are essential.[1–3] The most common finding in prenatal LQTS is a foetal heart rate (FHR) < 3rd percentile for gestational age (GA) in the late second and third trimesters.[4–6] It has been suggested that identifying a diagnostic FHR threshold might improve LQTS ascertainment before birth.[4,5] However, some of the data describing the FHR/GA relationship of LQTS patients were derived from a Swedish founder (SF) cohort of foetuses with LQTS type 1 (LQT1)[5] and potentially not generalizable to other LQT1 cohorts or to the two other major LQTS genotypes [LQTS type 2 (LQT2) and LQTS type 3 (LQT3)]. Furthermore, in the SF cohort, the specificity of a FHR threshold <3rd percentile was >97%, but the sensitivity was <50%.[4] Data from a LQT1, LQT2, and LQT3 cohort were heavily weighted with *de novo* LQT3 foetuses, whose FHRs, we now know, are

considerably lower than the FHRs of patients with familial variants.[4,6–8] Until now, the potential effects of pathogenic variants within LQTS genotypes were explored only partially,[9,10] and data on the effect of maternal β-blocker treatment on FHR were conflicting.

Because of these limitations, we sought to expand and refine the FHR/GA profile in familial (not *de novo*) LQTS pregnancies, taking cohort, genotype, familial variant, and maternal β-blocker therapy into consideration. We hypothesized that the FHR/GA profile would differ based on LQTS genotype, familial variant, and maternal β-blocker exposure. To address our hypothesis, we leveraged the standard of care genetic testing for offspring born to families with maternal or paternal LQTS, postnatally ascertaining genotype status, i.e. genotype-positive or genotype-negative, for the familial LQTS variant. We then compared the FHR/GA profile between genotype-positive and genotype-negative foetuses from two LQT1 founder populations and a multi-centre international cohort including the three canonical LQTS genotypes (LQT1, LQT2, and LQT3). We also assessed the association between FHR and the familial variant, defined as the pathologic or likely pathologic inherited variant, and between FHR and β-blocker exposure. Taking the results of these factors into account, we developed a FHR/GA model for the *in utero* prediction of LQTS in the context of a maternal/paternal pathogenic or likely pathogenic LQTS− variant.

Methods

Research cohort

This was a multi-centre retrospective review of pregnancies in LQT1, LQT2, and LQT3 families. Pregnancies were recruited from multiple sources including the website fetallqts.com, the Sudden Arrhythmic Death Syndromes (SADS) Foundation, and 12 international sites collectively known as the Fetal LQTS Consortium. The participating sites were in Sweden, the USA, The Netherlands, Finland, Norway, Germany, Italy, and Japan. Participating sites had interest and expertise in inherited heart rhythm disorders and included foetal cardiologists, paediatric and adult cardiologists and electrophysiologists, high-risk pregnancy specialists, and geneticists. The QT interval was measured,[11] and QTc was calculated according to Bazett's correction which is valid also in newborns.[12] Participating sites reviewed their clinical databases and sent letters to eligible subjects or discussed the study during the subject's visits to that site's inherited arrhythmia clinic. Inclusion criterion was an identified putative pathogenic LQTS variant in *KCNQ1* (LQT1), *KCNH2* (LQT2), or *SCN5A* (LQT3), in a mother who was currently or had previously been pregnant, or in her partner. Genotype positive was defined as heterozygous carriership of a likely pathogenic or pathogenic variant in *KCNQ1*, *KCNH2*, or *SCN5A* [according to American College of Medical Genetics and Genomics (ACMG) variant classification as of 1 May 2023]. Collected data related to LQT1–3 variants not fulfilling the current criteria for likely pathogenic or pathogenic were excluded from analyses and presented separately as Supplementary material. Further exclusion criteria were an unknown neonatal genotype, or a neonatal genotype homozygous or compound heterozygous for LQTS. Subjects were recruited from all participating consortium sites and included two Swedish LQT1 founder populations (SF, *KCNQ1*/p. Y111C and *KCNQ1*/p. R518*). 'LQT1' refers to both SF and consortium LQT1 cohorts combined.

The study was approved by the ethics committees or institutional review boards at each of the sites (CORE site UC-Denver IRB# 13-1879). The study was registered on ClinicalTrials.gov (NCT02876380). Pregnancy outcomes of this study cohort have been previously reported.[13]

Data collection

We collected parental, foetal, and postnatal data, including the cohort (consortium or SF), the parental and neonatal genotype (LQT1, LQT2, or LQT3), the specific LQTS variant, maternal or paternal origin of the LQTS variant, and maternal β-blocker therapy during pregnancy (yes or no). Foetal heart rate was measured during obstetrical visits throughout the pregnancy; data were preferentially derived by averaging three Doppler readings or three cardiac cycle lengths during foetal Doppler ultrasound when the foetus was quiet. We recorded GA in weeks and days for each obstetrical visit and grouped FHR data according to trimester: 1st trimester (<16 weeks GA), 2nd trimester (16–28 weeks GA), or 3rd trimester (29–42 weeks GA).

Genetic testing for the familial variant was performed as standard of care in a laboratory adhering to Clinical Laboratory Improvement Amendments (CLIA) after birth. Fetal LQTS status was defined as either positive or negative for the family's LQTS-causative variant (see Supplementary material online, *Table S1*).

Statistical analysis

Descriptive summaries are provided as mean (standard deviation) for continuous measures and as frequency (%) for categorical measures. To determine if SF and consortium LQT1 cohorts needed to be evaluated as separate groups or could be combined, linear mixed effects regression models were fit with a random intercept term for foetuses nested within a family. Covariates included cohort status, GA, maternal β-blocker exposure, and foetal LQTS status based on genetic testing performed postnatally. A quadratic term was included for GA based upon evaluating model fit and assumptions. A significant difference in the cohort would warrant the evaluation of LQT1 cohorts separately in subsequent models.

Models that included all LQTS genotypes used LQTS genotype (LQT1, LQT2, and LQT3) in place of cohort status. Graphical summaries of FHR present the mean and 95% confidence interval (CI) for the 2nd and 3rd trimesters as estimated from linear mixed effects regression models including a random intercept for foetus nested within family and stratified to the LQTS genotype. These models were not used for evaluating the statistical association but to simplify the presentation of the average trajectories across trimesters while accounting for the correlated nature of the data.

To determine whether there were genotype–phenotype correlations between LQTS variants and FHR within each LQTS subgroup, previously published predictors of severity were assessed.[9,14–17] Severity predictors included the ACMG variant classification, variant type (missense/nonsense/other), variant location (pore/non-pore, *KCNH2* variants only), functional loss of β-adrenergic response [protein kinase A (PKA)-dependent slow delayed rectifier potassium current (I_{Ks}) enhancement, *KCNQ1* variants only], and variant effects on I_{Ks} and rapid delayed rectifier potassium current (I_{Kr}) current density derived from functional studies (*KCNQ1* and *KCNH2* variants). Severe loss-of-function variants were defined as those causing impairment to β-adrenergic response and/or strong dominant-negative effects on current density (i.e. ≤25% of wild type (WT)). All comparisons used linear mixed effects regression models, including a random intercept for foetus nested within family.

To determine if our data could improve upon the *a priori* 50% transmission risk of LQTS genotype and anticipate/suggest foetal LQTS, statistical models for the testing algorithms to predict LQTS status were fit with mixed effects logistic regression models with predictors for FHR and GA. Using only FHR and GA, we derived a testing algorithm using GA as a continuous predictor to estimate the probability that a foetus was LQTS positive from a logistic regression model, with the 'optimal' probability threshold identified using Youden's *J* statistic to maximize combined sensitivity and specificity. The accuracy, sensitivity, and specificity of the 'optimal' model was compared to a naïve threshold of the obstetrical definition of bradycardia (<110 b.p.m. throughout gestation) and the previously proposed strategy using a FHR <3rd percentile to predict LQTS (1). All figures, summaries, and linear mixed effects regressions used R v4.1.0 (Vienna, Austria); the logistic mixed effects regression was fit using PROC GLIMMIX in SAS 9.4 (Cary, NC), with the residual pseudo-likelihood technique and the Newton–Raphson ridge optimization for estimation.

Results

Summary characteristics of eligible pregnancies

Data were obtained from 267 pregnancies resulting in 267 offspring from 164 families in which one parent (113 mothers and 51 fathers) had genetically confirmed LQTS: LQT1 (*KCNQ1*, 82%), LQT2 (*KCNH2*, 13%), or LQT3 (*SCN5A*, 5%) (*Table 1*). Of the 267 offspring, 158 were born to families from a SF cohort of LQT1 (*KCNQ1*/p.Y111C or *KCNQ1*/p. R518*), and 109 were delivered to families from an international LQTS

Table 1 Summary characteristics for eligible pregnancies by data source expressed as *N* (%)

Covariate	Pregnancies (*N* = 267)	Swedish founder (*N* = 158)	LQTS consortium (*N* = 109)
LQTS+ father	88 (33.0%)	60 (38.0%)	28 (25.7%)
LQTS+ mother	179 (67.0%)	98 (62.0%)	81 (74.3%)
LQT1	220 (82.4%)	158 (100.0%)	62 (56.9%)
LQT2	35 (13.1%)	0 (0.0%)	35 (32.1%)
LQT3	12 (4.5%)	0 (0.0%)	12 (11.0%)
LQTS+ foetus	159 (59.6%)	90 (57.0%)	69 (63.3%)
LQTS− foetus	108 (40.4%)	68 (43.0%)	40 (36.7%)

LQTS+, long QT syndrome positive status; LQT1, long QT syndrome type 1; LQT2, long QT syndrome type 2; LQT3, long QT syndrome type 3; LQTS−, long QT syndrome negative status.

Table 2 Summary characteristics for eligible pregnancies by foetal LQTS status expressed as *N* (%)

Covariate	Overall (*N* = 267)	LQTS+ foetus (*N* = 159)	LQTS− foetus (*N* = 108)
LQTS+ mother	179 (67.0%)	108 (67.9%)	71 (65.7%)
Maternal β-blocker	83 (46.4%)	54 (50.0%)	29 (40.8%)
SF LQT1	98 (54.7%)	54 (50.0%)	44 (62.0%)
Cons LQT1	140 (78.2%)	29 (26.9%)	13 (18.3%)
LQT2	31 (17.3%)	20 (18.5%)	11 (15.5%)
LQT3	8 (4.5%)	5 (4.6%)	3 (4.2%)
LQTS+ father	88 (33.0%)	51 (32.1%)	37 (34.3%)
SF LQT1	60 (68.2%)	36 (70.6%)	24 (64.9%)
Cons LQT1	80 (90.9%)	12 (23.5%)	8 (21.6%)
LQT2	4 (4.5%)	1 (2.0%)	3 (8.1%)
LQT3	4 (4.5%)	2 (3.9%)	2 (5.4%)

Cons LQT1, data from long QT syndrome type 1 consortium sample; LQT2, long QT syndrome type 2; LQT3, long QT syndrome type 3; LQTS+, long QT syndrome positive status; LQTS−, long QT syndrome negative status; SF LQT1, data from long QT syndrome type 1 Swedish founder cohort.

consortium of LQT1, LQT2, and LQT3 (*Table 1*). A total of 60% (159) of the 267 offspring inherited the mother or father's LQTS-causative variant (*Table 2*).

We evaluated maternal β-blocker treatment per pregnancy (83 out of 179 pregnancies with maternal LQTS) (*Tables 1 and 2*). Fewer LQTS mothers from the SF cohort received β-blockers when compared to the consortium [16% (16/98) vs. 83% (67/81)]. Forty-six per cent (83/179) of LQTS mothers in the combined cohorts were treated with β-blockers during their pregnancies: 40% (56/140) with LQT1, 84% (26/31) with LQT2, and 12% (1/8) with LQT3.

We evaluated 1966 FHRs (1265 from the SF cohort and 701 from the consortium cohort) at GA 7–42 weeks: 68 in the 1st trimester, 533 in the 2nd trimester, and 1365 in the 3rd trimester (see Supplementary material online, *Table S2*).

Effect of long QT syndrome type 1 cohort on foetal heart rate

After fitting a linear mixed effects regression model accounting for GA, maternal β-blocker use, and the interaction between β-blocker use and LQT1 status of the foetus, the mean FHRs of the LQT1 foetuses were not significantly different between the SF and the consortium cohorts (*Table 3*, $P = 0.069$). The data for the specific founder variant types p.Y111C (missense, dominant negative) and p.R518* (nonsense, haploinsufficiency) are included in the analysis and presented in *Table 4* and Supplementary material online, *Table S3*. These findings suggest the LQT1 foetuses can be analysed by genotype, without accounting for the cohort source, in subsequent models.

Effect of long QT syndrome genotype on foetal heart rate

An overview of the FHR data, stratified by LQTS genotype, is shown as a continuous trend across gestation in *Figure 1*. As noted in previous studies,[3,4] the mean FHR differed significantly between LQTS genotype-positive and LQTS genotype-negative foetuses ($P < 0.001$). However, there was not a significant FHR difference between LQT1 and LQT2 genotype-positive FHRs [−0.3 b.p.m. (95% CI: −3.0 to 2.5 b.p.m.; $P = 0.842$)].

An unexpected and previously unreported observation was that the FHRs of LQT3 genotype-positive foetuses were significantly higher on average than FHRs of LQT1 genotype-positive foetuses [+5.4 b.p.m. (95% CI: 1.2–9.6 b.p.m.; $P = 0.011$)] and LQT2 genotype-positive foetuses [+5.7 b.p.m. (95% CI: 0.9–10.5 b.p.m.; $P = 0.021$)] (*Figure 2*). These findings already accounted for the use of maternal β-blocker treatment through the adjusted regression modelling. Significantly, the FHR/GA profiles of those with familial LQT3 were in stark contrast to the FHR/GA profiles of the previously published cohort of foetuses with *de novo* LQT3, whose FHRs were markedly lower.[5,6–8]

Effect of long QT syndrome variants on foetal heart rate

Among LQT1 genotype-positive foetuses ($n = 131$) (*Table 2*), 7 had a familial variant classified as likely pathogenic, and 124 had a familial variant classified as pathogenic. There was no significant association between variant class and FHR among LQT1 genotype-positive foetuses ($P > 0.05$). However, as a novel finding, among LQT1 genotype-positive foetuses, variant types with potentially greater loss of I_{Ks} were significantly associated with lower FHRs (missense vs. nonsense, $P = 0.003$; non-missense vs. nonsense, $P = 0.027$, *Table 4*; Supplementary material online, *Table S3*). Moreover, LQT1 variants with severe function loss (current density ≤25% of WT or impaired β-adrenergic response) had lower FHRs compared to haploinsufficiency-causing nonsense variants ($P = 0.012$, *Table 4* and *Figure 3*; Supplementary material online, *Table S3*).

Among LQT2 genotype-positive foetuses ($n = 21$), 6 had a familial variant classified as likely pathogenic or novel frameshift, and 15 had a familial variant classified as pathogenic. There was no significant association between variant class and FHR among LQT2 genotype-positive foetuses ($P > 0.05$). No significant associations were seen between variant types with potentially greater loss of I_{Kr} and FHR in the LQT2 foetuses (pore vs. non-pore variants, $P > 0.05$; dominant-negative vs. haploinsufficiency-causing variants, $P > 0.05$, *Table 4*; Supplementary material online, *Table S3*).

Among LQT3 genotype-positive foetuses ($n = 7$), one had a familial variant classified as likely pathogenic, and six had a familial variant classified as pathogenic. No association analyses were performed due to the small LQT3 sample size.

During the study, data were also collected on 15 pregnancies in LQTS families with a LQT1–3 variant not fulfilling the classification criteria of likely pathogenic or pathogenic. Data related to these

Table 3 Mean (SD) [number of observations] FHR by trimester based on postnatal LQTS status and genotype for SF and consortium cohorts

Data source and trimester	LQT1+ FHR	LQT1− FHR	LQT2+ FHR	LQT2− FHR	LQT3+ FHR	LQT3− FHR
All data: 1st	151 (12) [36]	155 (7) [13]	141 (7) [11]	152 (15) [7]	150 (–) [1]	ND
All data: 2nd	135 (11) [282]	147 (6) [164]	135 (7) [30]	144 (10) [32]	149 (8) [11]	150 (6) [14]
All data: 3rd	132 (11) [725]	142 (8) [486]	127 (11) [71]	140 (10) [48]	139 (5) [17]	143 (7) [18]
SF LQTS: 1st	128 (15) [3]	159 (7) [3]	ND	ND	ND	ND
SF LQTS: 2nd	137 (8) [195]	147 (6) [122]	ND	ND	ND	ND
SF LQTS: 3rd	134 (10) [536]	143 (8) [406]	ND	ND	ND	ND
Consortium: 1st	153 (10) [33]	154 (6) [10]	141 (7) [11]	152 (15) [7]	150 (–) [1]	ND
Consortium: 2nd	130 (14) [87]	146 (6) [42]	135 (7) [30]	144 (10) [32]	149 (8) [11]	150 (6) [14]
Consortium: 3rd	126 (13) [189]	138 (8) [80]	127 (11) [71]	140 (10) [48]	139 (5) [17]	143 (7) [18]

FHR, foetal heart rate; LQTS, long QT syndrome; LQT1, long QT syndrome type 1; LQT2, long QT syndrome type 2; LQT3, long QT syndrome type 3; –, negative; ND, no data; +, positive; SF, Swedish founder cohort; SD, standard deviation.

Table 4 The effect on foetal heart rate in beats per minute by severity predictors fit with linear mixed effects model including nested random intercepts for pregnancies within LQT1 and LQT2 families

Covariate	β-Estimate (95% CI)	*P*-value
KCNQ1: variant type		
Missense (vs. nonsense)	−7.0 (−11.5, −2.5)	0.003
Other/non-missense (vs. nonsense)	−12.1 (−22.5, −1.6)	0.027
KCNQ1: current density ≤25% of wild type or impaired β-adrenergic response		
Severe function loss (vs. haploinsufficiency)	−5.83 (−10.23, −1.44)	0.012
KCNH2: variant type		
Pore variant (vs. non-pore variant)	1.0 (−8.8, 10.6)	0.848
KCNH2: current density ≤25% of wild type		
Severe function loss (vs. haploinsufficiency)	−3.5 (−14.8, 7.7)	0.558

pregnancies were not included in the analyses or modelling but are presented in Supplementary material online, *Table S4A–C*.

Effect of maternal β-blocker on foetal heart rate

Eighty-three LQTS genotype-positive mothers received β-blockers and delivered 54 LQTS genotype-positive and 29 LQTS genotype-negative infants (*Table 2*; Supplementary material online, *Table S5A–C*). Long QT syndrome genotype-positive foetuses exposed to β-blockers had the lowest FHRs in the study population (*Figure 4* and *Table 5*). Compared to FHRs of LQTS genotype-positive foetuses from untreated mothers, β-blocker-exposed LQTS genotype-positive FHRs were lower by 9.9 b.p.m. (95% CI: 7.6–12.3 b.p.m., $P < 0.001$). However, among the LQTS genotype-negative foetuses, exposure to maternal β-blocker therapy did not significantly affect FHR. Specifically, LQTS genotype negative foetuses whose mothers were on β-blocker therapy had an average FHR that was 2.3 b.p.m. lower than genotype negative foetuses not exposed to β-blockers (95% CI: 5.4 b.p.m. lower to 0.7 b.p.m. higher; $P = 0.13$). Moreover, in pregnancies not treated with β-blockers (184/267), FHRs of LQTS genotype-positive foetuses remained significantly lower than FHRs of LQTS genotype-negative foetuses by 7.8 b.p.m. (95% CI: 6.0–9.7 b.p.m., $P < 0.001$) after adjusting for GA and LQTS genotype. Thus, maternal β-blocker treatment appeared to potentiate, rather than cause, the lower intrinsic FHR of LQT1 and LQT2 genotype-positive foetuses.

Foetal heart rate/gestational age profiles to identify long QT syndrome type 1 and long QT syndrome type 2 genotype-positive foetuses

The testing algorithms that we developed and evaluated are restricted to LQT1 and LQT2 since the sample size of LQT3 was small and there was little difference in FHR between LQT3 genotype-positive and LQT3 genotype-negative foetuses (*Tables 6* and *7* and *Figure 4*). Using the traditional model defining foetal bradycardia as FHR <110 b.p.m. would successfully identify all the LQT1 and LQT2 genotype-negative foetuses as normal, but only 12/152 (7.9%) of LQT1 and LQT2 genotype-positive foetuses as affected. This would result in accuracy, sensitivity, and specificity of 45.1, 7.9, and 100%, respectively. Importantly, 92% (140/152) of LQT1 and LQT2 genotype-positive cases would have been misclassified as normal. Assuming a prevalence of 50% for inheriting a LQTS variant, the positive predictive value (PPV) is 100%, but the negative predictive value (NPV) is only 52.1% for a strict FHR <110 b.p.m. threshold.

In contrast, we can use these data to validate the previously proposed definition of FHR <3rd percentile for GA to identify the presence of either foetal LQT1 or LQT2. A total of 129/152 (84.9%) of LQT1 and LQT2 genotype-positive foetuses had FHRs <3rd percentile for GA, giving an accuracy, sensitivity, and specificity of 74.5, 84.9, and 59.2%, respectively. The lower specificity means 41% of those genotype negative for their parent's LQT1/LQT2-causative variant would have been misclassified as genotype positive. Further, assuming a 50% prevalence for inheriting the LQTS variant, the PPV and NPV are 67.5 and 79.6%, respectively, for this approach.

The testing algorithm developed in this study using GA as a continuous predictor to estimate the probability that a foetus is LQTS genotype positive from a logistic regression model had the best results when trying to balance both sensitivity and specificity. Overall, using the largest predicted probability for each foetus with this model resulted in an area under the

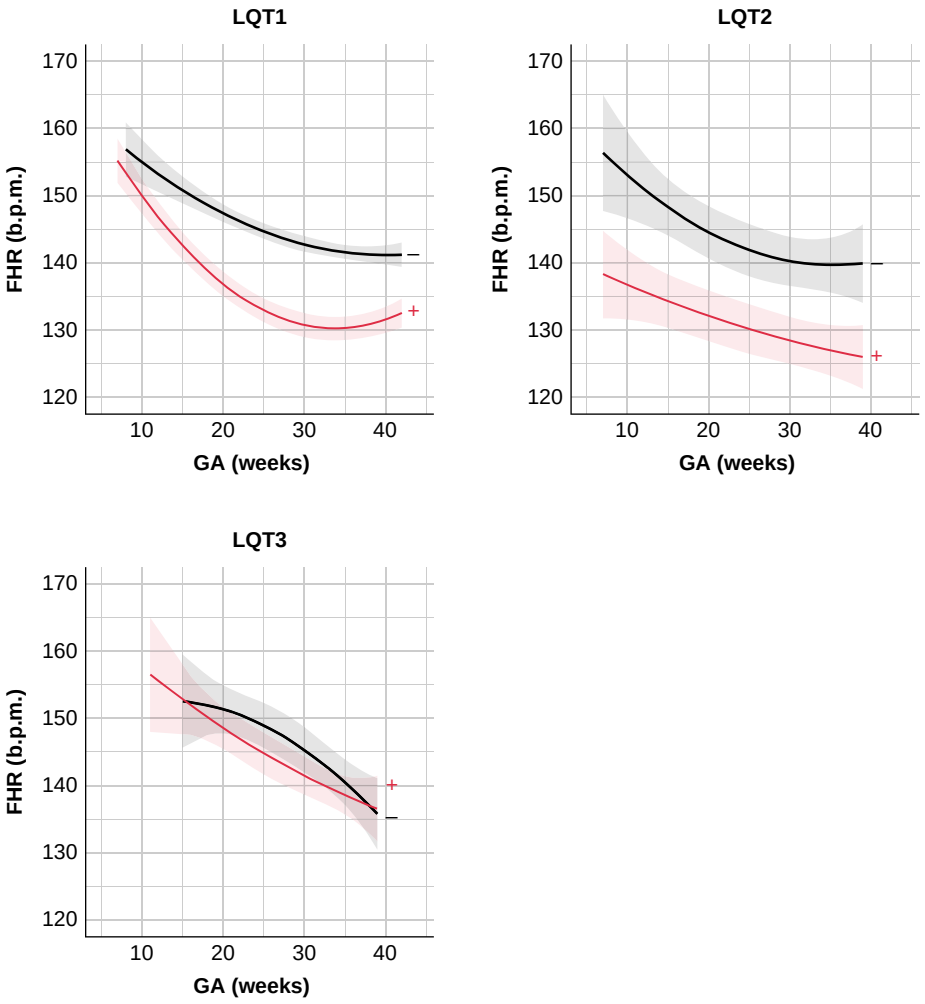


Figure 1 Foetal heart rate across gestational age (GA) by long QT syndrome (LQTS) genotype. Based on our data, this figure depicts the continuous trend of fetal heart rate (FHR), modelled across gestation for genotype-negative (–) and genotype-positive (+) foetuses born to parents with long QT types 1, 2 and 3 (LQT1, LQT2, or LQT3) genotype, including 95% CI. The FHR differences between genotype (+) and genotype (–) LQTS subjects vary across gestation and between LQTS genotypes: increasing and then stabilizing in LQT1, remaining constant in LQT2, and without meaningful differences in LQT3.

curve (AUC) of 81.6% (95% CI: 76.4–86.8%). Specifically, using a predicted probability >83.2% to predict LQTS results in an overall accuracy of 74.9%, with an overall true positive rate (sensitivity) of 71.1% and an overall true negative rate (specificity) of 80.6%. This indicates that 28.9% of LQTS genotype-positive foetuses would be incorrectly predicted as LQTS genotype negative while 19.6% of LQTS genotype-negative foetuses would be incorrectly predicted to have LQTS. Given an *a priori* 50% genetic transmission risk, the PPV for this strategy is 78.5%, which indicates that if the FHR is below the threshold, there is a 78.5% probability that the foetus will be LQTS genotype positive. The NPV for this strategy is 73.6%, indicating that FHR above the GA-specific threshold indicates a 73.6% chance the foetus is truly LQTS genotype negative. The newly derived FHR thresholds by GA are presented in *Table* 7. Relative to the <3rd percentile model, this approach better balances the true positive and negative rates and better anticipates a foetal diagnosis of LQTS compared to the background point estimate of 50%.

Discussion

The present data, from what is the largest study of familial LQTS describing the effects of cohort, genotype, familial variant, and *in utero* exposure to maternal β-blocker therapy on the FHR of LQTS genotype-positive and LQTS genotype-negative foetuses, provide several novel findings important in perinatal LQTS ascertainment. Firstly, LQT1 FHR data seem generalizable as SF and consortium cohort data are comparable. Secondly, LQTS genotype affects FHR, as shown by the fact that LQT1 and LQT2 (but not LQT3) FHRs are lower than their LQTS genotype-negative counterparts. Moreover, among LQT1 genotype-positive foetuses, both variant type (missense/nonsense) and the functional effects of variants on current density and β-adrenergic response are associated with FHR, with the lowest FHRs found in foetuses with non-nonsense variants and variants with severe function loss. Thirdly, in β-blocker-treated pregnancies, the intrinsically lower FHRs in LQT1 and LQT2 genotype positives are potentiated, while FHRs in LQTS genotype negatives are not significantly affected. Lastly, for identifying foetuses with potassium channel–mediated LQTS as early as 14–16 weeks of gestation, we found that the overall accuracy of the current FHR <3% and of our proposed thresholds was similar (74.5% for <3rd percentile and 74.9% for proposed threshold). There are different strengths for each strategy: the proposed threshold better balanced sensitivity and specificity (71.1 and 80.6%, respectively), whereas the <3rd percentile has higher

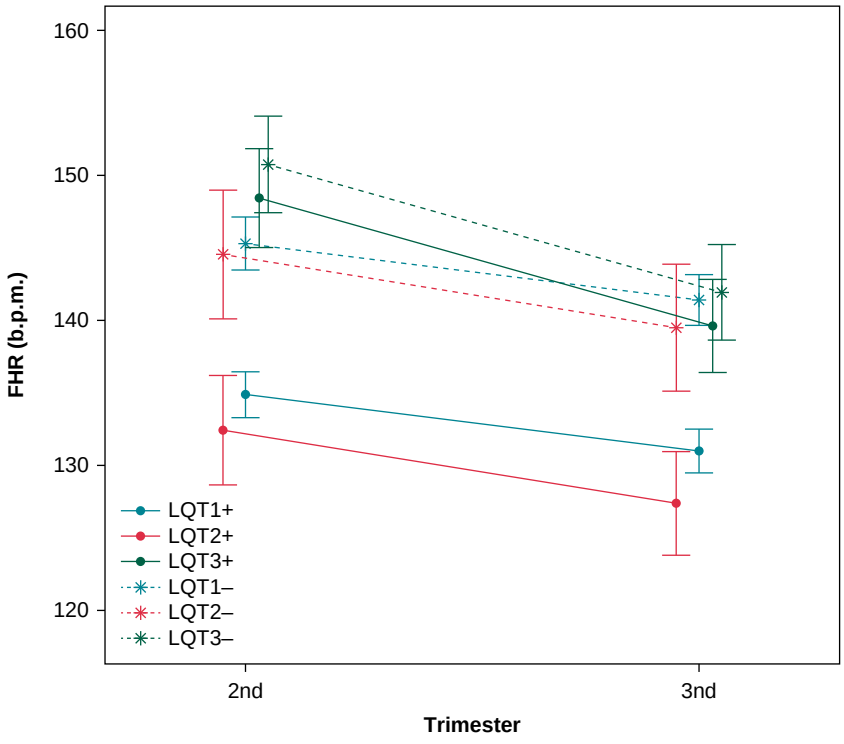


Figure 2 Mean fetal heart rate (FHR) by trimester based on foetal long QT syndrome (LQTS) status and genotype presented as mean (95% CI) estimated from a linear mixed effects model to account for the repeated measures during a pregnancy.

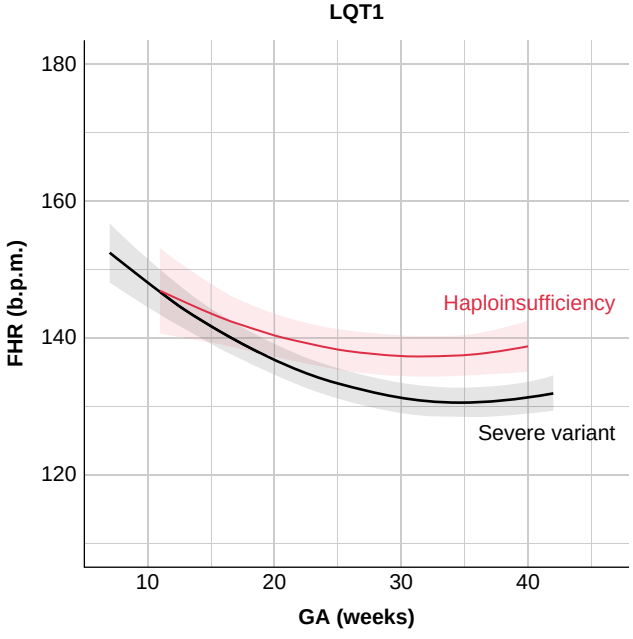


Figure 3 Foetal heart rate (FHR) across gestational age (GA) by long QT type 1 (LQT1) haploinsufficiency and severe variant classification. Based on our data, this figure depicts the continuous trend of FHR by each subgroup.

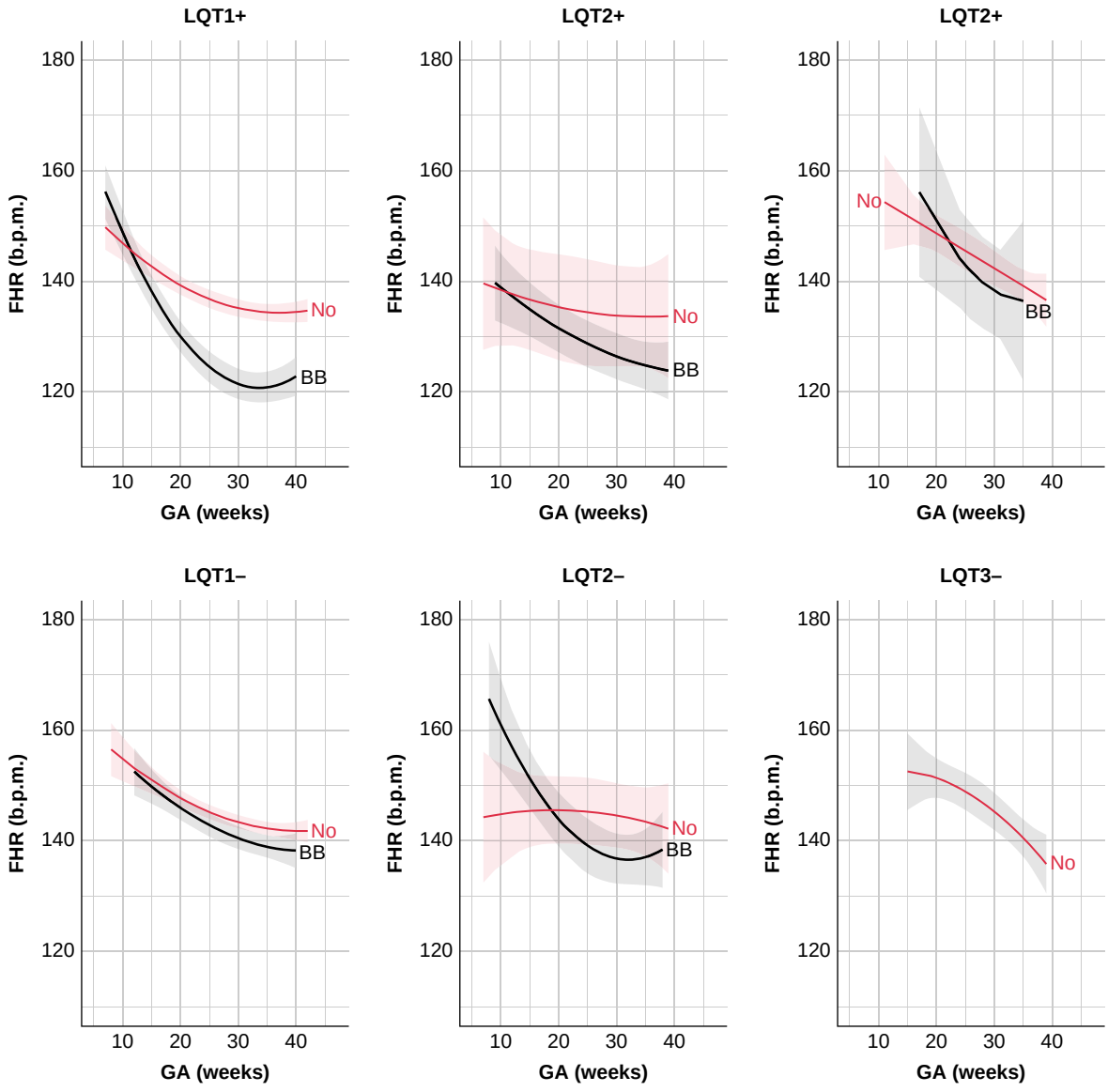


Figure 4 Foetal heart rate (FHR) across gestational age (GA) by long QT syndrome (LQTS) genotype and maternal β-blocker therapy use. Based on our data, this figure depicts the continuous trend of FHR by each subgroup.

sensitivity but lower specificity (84.9% sensitivity and 59.2% specificity). This suggests that if higher specificity or more balance between sensitivity and specificity is desired, the proposed thresholds are preferable; otherwise, if only higher sensitivity is desired, the <3rd percentile thresholds is superior.

The association of LQTS and 'moderate' foetal bradycardia was known as early as 1995[18] but not characterized as FHR <3rd percentile for GA until 2012.[4] In 2015, the distinction of LQTS bradycardia as <3rd percentile for GA rather than the traditional definition of FHR <110 b.p.m. was corroborated in 110 LQT1 foetuses from two SF populations.[5] We did not find a difference in FHRs between the LQT1 SF and consortium cohorts. In both cohorts, FHR successfully distinguished between LQT1 genotype-positive and LQT1 genotype-negative foetuses.

The 2012 study on FHR in LQTS included only 23 LQT1, 4 LQT2, and 6 LQT3 foetuses, and 5/6 of the LQT3 foetuses had *de novo* variants resulting in severe LQT3 expressivity.[4] In only one foetus was the LQT3 variant inherited. Thus, this is the first study to include inherited LQT3 variants and to demonstrate that not only are LQT3 FHRs higher than LQT1 and LQT2 FHRs, but importantly, FHR does not appear to distinguish between LQT3 genotype-positive and LQT3 genotype-negative foetuses. It is somewhat surprising that FHRs were higher in LQT3 than in LQT1 and LQT2, given that sinus bradycardia is a marker of LQT3 in adolescence and young adulthood.[19] This study raises the question whether sinus bradycardia might be a phenotype associated with *de novo* LQT3, but not familial LQT3, also after birth.

As a novel finding, in the combined LQT1 cohort, there was a significant association between FHR and variant type, where those with non-nonsense and LQT1 variants severely affecting *KCNQ1* function had lower FHRs than those with the milder, haploinsufficiency-causing variants. This suggests that the familial variant within the LQT1 genotype affects the severity of the FHR phenotype. A genotype–phenotype

Table 5 Mean (SD) [number of observations] FHR by trimester, LQTS genotype/status, and β-blocker exposure

Trimester	LQT1– no BB	LQT1– BB	LQT2– no BB	LQT2– BB	LQT3– no BB	LQT3– BB
1	156 (7) [9]	152 (5) [4]	139 (15) [3]	162 (4) [4]	ND	ND
2	147 (7) [127]	146 (6) [37]	146 (10) [16]	143 (9) [16]	150 (6) [14]	ND
3	143 (8) [404]	139 (7) [82]	142 (7) [20]	138 (11) [28]	143 (7) [18]	ND
Trimester	**LQT1+ no BB**	**LQT1+ BB**	**LQT2+ no BB**	**LQT2+ BB**	**LQT3+ no BB**	**LQT3+ BB**
1	148 (16) [14]	152 (9) [22]	138 (6) [7]	147 (2) [4]	150 (–) [1]	ND
2	137 (9) [212]	126 (10) [70]	137 (9) [7]	134 (6) [23]	149 (8) [8]	149 (10) [3]
3	135 (10) [561]	122 (10) [164]	131 (14) [14]	126 (10) [57]	140 (5) [14]	137 (5) [3]

BB, β-blocker; LQT1, long QT syndrome type 1 (combined consortium and Swedish founder cohorts); LQT2, long QT syndrome type 2; LQT3, long QT syndrome type 3; –, genotype negative; ND, no data; +, genotype positive; SD, standard deviation.

Table 6 Comparison of three models predicting LQTS genotype-positive and LQTS genotype-negative foetuses

Model	True LQTS –	True LQTS +	False LQTS –	False LQTS +	Accuracy	Sensitivity	Specificity	Positive predictive value (%)[a]	Negative predictive value (%)[a]
FHR < 110 b.p.m.	103	12	140	0	45.1% (115/255)	7.9% (12/152)	100.0% (103/103)	100.0	52.1
FHR < 3rd % for GA	61	129	23	42	74.5% (190/255)	84.9% (129/152)	59.2% (61/103)	67.5	79.6
Current FHR/GA threshold model	83	108	44	20	74.9% (191/255)	71.1% (108/152)	80.6% (83/103)	78.5	73.6

FHR, foetal heart rate; GA, gestational age; LQTS, long QT syndrome; –, genotype-negative; +, genotype-positive.
[a]Prevalence of 50% for inheriting LQTS assumed for predictive value calculation.

correlation between *KCNQ1* function loss and FHR has been described previously in the studies by Winbo *et al.*[5] and Winbo and Rydberg,[20] where foetuses with homozygous or compound heterozygous *KCNQ1* variants had significantly lower FHRs compared to their heterozygous siblings and relatives.

Fetal bradycardia in LQTS pregnancies has long been attributed to maternal β-blocker treatment, but data are limited and often do not apply to currently recommended treatments for LQTS. For example, the mean FHR dropped from 144 to 133 b.p.m. among hypertensive pregnant women treated with 100 mg/day of atenolol[21]; however, atenolol is not recommended in pregnancy as it is associated with significant foetal growth restriction.[22] Metoprolol had no abnormal effects on the foetuses in 101 treated hypertensive women[23]; however, due to the higher recurrence of cardiac events in metoprolol-treated LQTS patients, other β-blockers, such as nadolol or propranolol, might be preferable.[24–26] Based on our data and experiences, and in line with the latest Heart Rhythm Society Expert Consensus Statement on the Management of Arrhythmias During Pregnancy,[27] we strongly recommend continuation of β-blocker therapy for LQTS mothers during pregnancy, with nadolol, propranolol, and metoprolol all being safe options from a foetal perspective.

Unfortunately, we did not have enough subjects with good quality data to determine a type-specific β-blocker effect on FHR, but our findings suggest a complex relationship. Our results differ from those of Winbo *et al.*, who found that maternal β-blockers lowered FHRs of both LQT1 genotype-positive and LQT1 genotype-negative foetuses in 21 treated pregnancies.[4] In contrast, our results were based on 83 treated pregnancies with LQT2 and LQT3 in addition to LQT1. The authors would like to clarify that the low percentage of maternal β-blocker treatment in the SF LQT1 cohort is largely due to access to pregnancy records from before the mothers were diagnosed with LQTS and does not reflect differences in clinical management of SF pregnant mothers.[5]

Study limitations

Although LQT1, LQT2, and LQT3 genotypes are represented, fewer inherited LQT3 patients were included. Similarly, we collected less 1st and 2nd trimester FHR data, as FHR is assessed monthly in the 1st and 2nd trimesters but weekly in the 3rd trimester. As mentioned previously, we did not collect sufficiently detailed data to discriminate specific maternal β-blocker effects on FHR, nor did we collect phenotypic data to understand why some pregnant subjects were treated and others were not. Data on postnatal QTc were asked for from each participating site; however, upon revision of the collected data, it was evident that (i) electrocardiograms were recorded at widely different ages, from just after birth to several months of age, and (ii) QTc measurements were not performed in a uniform way. Ultimately, the postnatal QTc data were deemed of too low quality to confidently include in this study. Finally, we did not collect data on the sex of the offspring; thus, we do not know if that might be a variable affecting our findings, as it is in postnatal LQTS. While we were able to validate the previously identified FHR <3rd percentile for GA as a predictor of LQTS genotype, future research will be needed to validate our

Table 7 Foetal heart rate/gestational age thresholds with strategy metrics for predicting LQT1 and LQT2

Trimester	GA (weeks)	FHR (b.p.m.)
2nd	14	136
	15–17	135
	18–20	134
	21–23	133
	24–26	132
3rd	27–29	131
	30–32	130
	33–35	129
	36–38	128
	39–41	127
	42	126
Strategy accuracy		74.9%
Strategy sensitivity		71.1%
Strategy specificity		80.6%
Strategy PPV[a]		78.5%
Strategy NPV[a]		73.6%

b.p.m., beats per minute; FHR, foetal heart rate; GA, gestational age; NPV, negative predictive value; PPV, positive predictive value.
[a]Assuming 50% prevalence.

proposed model which better balanced the sensitivity and specificity of prenatal LQTS prediction.

Conclusions

This large multi-centre study of prenatal familial LQTS demonstrates the difficulty of assigning an overarching FHR phenotype to LQTS subjects before birth and provides natural history data for LQTS offspring in the three most common LQTS genotypes. Although the FHRs of LQT3 genotype-positive foetuses in this cohort were indiscernible from those not inheriting the familial variant, we have derived a new FHR/GA predictive model that improves the diagnostic likelihood of inherited foetal LQTS types 1 and 2.

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: M.J.A. is a consultant for Abbott, Boston Scientific, Bristol Myers Squibb, Daiichi Sankyo, Invitae, Medtronic, Tenaya Therapeutics, and UpToDate. M.J.A. and Mayo Clinic are involved in equity/intellectual property/royalty relationships with AliveCor, Anumana, ARMGO Pharma, Pfizer, and Thryv Therapeutics. A.A.M.W. is a consultant for Thryv Therapeutics with minor financial interest. S.P.E. is involved with Spaulding Research with minor financial interest.

Data availability

Data are available upon reasonable request to the corresponding author.

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