Original Article

Third Trimester Fetal Heart Rate Predicts Phenotype and Mutation Burden in the Type 1 Long QT Syndrome

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Background—Early diagnosis and risk stratification is of clinical importance in the long QT syndrome (LQTS), however, little genotype-specific data are available regarding fetal LQTS. We investigate third trimester fetal heart rate, routinely recorded within public maternal health care, as a possible marker for LQT1 genotype and phenotype.

Methods and Results—This retrospective study includes 184 fetuses from 2 LQT1 founder populations segregating p.Y111C and p.R518X (74 noncarriers and 110 KCNQ1 mutation carriers, whereof 13 double mutation carriers). Pedigree-based measured genotype analysis revealed significant associations between fetal heart rate, genotype, and phenotype: mean third trimester prelabor fetal heart rates obtained from obstetric records (gestational week 29–41) were lower per added mutation (no mutation, 143±5 beats per minute; single mutation, 134±8 beats per minute; double mutations, 111±6 beats per minute; P<0.0001), and lower in symptomatic versus asymptomatic mutation carriers (122±10 versus 137±9 beats per minute; P<0.0001). Strong correlations between fetal heart rate and neonatal heart rate (r=0.700; P<0.001) were found. In a multivariable model, fetal genotype explained the majority of variance in fetal heart rate (−10 beats per minute per added mutation; P<1.0×10⁻²³). Arrhythmia symptoms and intrauterine β-blocker exposure each predicted −7 beats per minute, P<0.0001.

Conclusions—In this study including 184 fetuses from 2 LQT1 founder populations, third trimester fetal heart rate discriminated between fetal genotypes and correlated with severity of postnatal cardiac phenotype. This finding strengthens the role of fetal heart rate in the early detection and risk stratification of LQTS, particularly for fetuses with double mutations, at high risk of early life-threatening arrhythmias. (Circ Arrhythm Electrophysiol. 2015;8:806-814. DOI: 10.1161/CIRCEP.114.002552.)

Key Words: arrhythmias, cardiac • genotype • genetic association studies • heart rate • long QT syndrome

Background

Long QT syndrome (LQTS) is an inherited arrhythmia syndrome incurring risk of sudden cardiac death early in life. The genetic background of LQTS is diverse, however, the most prevalent genetic mutations occur in the KCNQ1, KCNH2, and SCN5A genes, corresponding to LQT1–3. LQTS prevalence has been estimated to 1:250 to 1:1000,8–11 and it has been reported that 5% to 10%3–5 of LQTS cases have a second mutation in the same or another LQTS susceptibility gene, leading to an increased risk for cardiac events. Double KCNQ1 mutations (homozygous or heterozygous) lead to a severe cardiac phenotype, when in combination with congenital deafness known as the Jervell and Lange–Nielsen syndrome (JLNS), a condition relatively more common in Scandinavia (1:200000) where carriership of LQTS mutations has been estimated to be between 1:250 and 1:1000.6–11

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Life-saving preventive therapies are available for LQTS patients, and as symptoms may occur from fetal life onwards, early detection or suspicion of diagnosis may be of great clinical importance. Prenatal rhythms associated with LQTS include torsade de pointes type ventricular tachycardia, 2:1 atrioventricular block, sinus bradycardia <110 beats per minute and a reduced baseline fetal heart rate (110–120 beats per minute), with the latter 2 being the most common. Indeed, neonatal sinus bradycardia has often been noted since the first reports on LQTS and JLNS. However, as fetal manifestations in LQTS have typically been described in cases presenting with fetal arrhythmia, it has not been established whether fetal heart rate in LQTS correlates with genotype per se. To date, the combined data on fetal heart rate in genotype ascertained LQTS is based on <50 mutation carriers, leading to a paucity of genotype-specific data. Moreover, little is known about whether relative bradycardia in utero correlates to postnatal cardiac phenotype in LQTS.

In this study, we take advantage of our large and relatively homogeneous Swedish LQT1 founder populations segregating the p.Y111C and p.R518X mutations to investigate whether fetal heart rate differs between LQT1 genotype groups (including noncarriers, carriers of single LQT1 mutations, and carriers of double LQT1 mutations). Furthermore,
WHAT IS KNOWN

• Sudden cardiac death may be the first presenting symptom in the long QT syndrome (LQTS), and early presymptomatic diagnosis and risk stratification is of clinical importance

• LQTS fetuses may present with signature rhythms in utero, including sinus bradycardia or a reduced fetal heart rate, and a fetal heart rate less than the third percentile for gestational age has been suggested as a potential clinical cutoff for LQTS suspicion.

WHAT THE STUDY ADDS

• In LQTS type 1, mean third trimester fetal heart rate may be used as a marker for mutation burden, as heart rates were lower per mutation carried (when compared between carriers of no, single, and double mutations), as well as for risk stratification, as fetal heart rates correlated with postnatal QTc and were associated with development of arrhythmia symptoms.

• Maternal β-blocker usage, associated with lower intrauterine fetal heart rates, was identified as a variable of interest when assessing fetal heart rate in LQTS families.

• Importantly, fetal heart rate, already monitored within standard maternal healthcare, shows promise to flag the LQTS fetuses at highest risk of life-threatening cardiac events.

we aim to investigate whether fetal heart recordings, obtained as part of standard medical care, have a predictive value for postnatal phenotype in these LQT1 populations.

Methods

Recruitment of Mothers and Fetuses

The study population was recruited from 2 large Swedish founder populations, segregating either p.Y111C16 (22 index families) or p.R518X11 (13 index families), where cascade-screening for KCNQ1 gene (LQT1) mutations had been previously performed in the clinical setting. The term family here indicates the first ascertained index gene (LQT1) mutations had been previously performed in the clinical setting. The term family here indicates the first ascertained index gene (LQT1) mutations had been previously performed in the clinical setting. The Regional Ethical Committee in Umeå, Umeå University, Sweden. The time frame (1980–2014) was chosen based on accessibility of standard maternal healthcare, shows promise to flag the LQTS fetuses at highest risk of life-threatening cardiac events.

Clinical Data

For each fetus, the following retrospective data were obtained from pedigrees and clinical records including maternal healthcare records: information about the mothers’ genotype and β-blocker usage, fetal sex, fetal genotype, fetal heart rate per gestational week, gestational age at birth, ECGs recorded at the time of diagnosis (when available), and data on arrhythmia symptoms associated with LQTS (defined as electrocardiographically verified arrhythmia, experience of syncope or cardiac arrest). Fetal heart rate recordings were obtained as part of standard medical care, typically once monthly during the second trimester and bimonthly during the third trimester, by Doppler ultrasonography or cardiotocography during fetal quiescence. The mean third trimester fetal heart rate per individual was calculated from all recordings, noted in the maternal healthcare records, from gestational week 29 and onwards, excluding recordings of tachycardia ≥200 beats per minute. The mean third trimester heart rate was used, as opposed to using data from the entire fetal period as (i) routine checkups are most frequent during this period and (ii) fetal heart rates are gestational age dependent and show a gradual decrease as gestation proceeds.18 Recordings of either fetal bradycardia (defined as fetal heart rate ≤110 beats per minute, obstetric standard) or tachycardia (≥200 beats per minute) at any time during gestation were noted for each fetus.

The first 12-lead ECG performed postnatally, typically recorded at 50 mm/s sweep speed, was obtained from medical records and measured manually by 1 observer. QT intervals were measured, preferably in lead II, as a mean of 3 consecutive QT intervals, and corrected for heart rate by Bazett’s formula (QT/√HR), using the mean of the R-R intervals preceding the measured beats. When available, neonatal heart rates (in beats per minute) were obtained from neonatal records or ECGs recorded during the first month of life. Neonatal ECGs were additionally evaluated in lead II for prevalent rhythm (sinus rhythm or atrioventricular block).

Statistical Analyses

Data were summarized and presented as total number plus percentage for proportions, and means±SD for continuous variables. Pearson correlations were calculated between continuous variables/covariates and results presented with correlation coefficient (r) and associated P value. Because of the nonindependence among family members in the 2 included founder populations,17 analyses of variance for single and combined covariates were performed using pedigree-based measured genotype association analysis in Sequential Oligogenic Linkage Analysis Routines (SOLAR)18 software (http://www.sphr.org/solar) and findings validated using Statistical Analysis for Genetic Epidemiology software (SAGE19; http://darwin.cwru.edu). To provide relatedness data for the SOLAR and SAGE software, pedigrees were constructed for each founder population, linking the nuclear families and family branches, via a best estimate approach based on available family pedigrees and previously published genealogical and microsatellite data for all included families.11,14,16,20

The measured genotype approach14,21 estimates genotype-specific trait means in large pedigrees by a fixed-effects model. To control for effects of multiple covariates, an initial maximum likelihood model was constructed in SOLAR (and validated using SAGE) for the primary trait mean third trimester heart rate, screening the covariates fetal genotype (no mutation=0, single mutation=1, double mutations=2), fetal sex (female=1, male=2), fetal phenotype (no arrhythmia symptoms=0, syncope and intrauterine arrhythmia=1), mothers’ genotype (noncarrier=0, mutation carrier=1), and intrauterine β-blocker exposure (no exposure=0, exposure to β-blockers=1) for significant association with the primary trait. The covariate QTc was omitted from the model construction as QTc values were only available for a subset of the sample (separate analyses for QTc were performed). A final restricted model comprising covariates with a P value <0.1 was subsequently constructed. For the final model, the polygenic ascertained by denaturing high-performance liquid chromatography (Wave 3500 HT, Transgenomic Inc, Omaha, Neb) and sequencing all coding exons of the KCNQ1 gene (CEQ 8000, Beckman Coulter, Fullerton, CA). In some probands, >1 KCNQ1 mutation were identified. Probands with a clinical JLNS diagnosis were also screened for additional mutations in the KCNE1 gene.8,11 In family members, mutation carriage was ascertained by sequencing or targeted mutation analysis of the identified mutations (MGB-probes by ABI 7000, Applied Biosystems, Foster City, CA).11,16

Genotype Ascertainment

As previously described,11,16 ascertainment of carrier status had been performed according to the clinical praxis, using genomic DNA extracted by a standard salting-out procedure, and genotypes in index cases had been...
heritability (H²: corresponding to the proportion of the phenotype variance in a trait that is attributable to the additive effects of genes) and associated P value, the residual kurtosis (within normal range [−0.8]) for all presented results, if not otherwise specified, the proportion of variance caused by the covariates and the covariates β coefficients were also calculated. The coefficients of the covariates (effect size) represent the values of the parameters in the model itself. For all analyses, a 2-tailed P value of <0.05 was considered statistically significant. Figures were constructed using Inkscape (Open Source software) and IBM SPSS Statistics 19.

Results
Study Population
The study included 184 pregnancies in 88 mothers (57 carriers of single LQT1 mutations and 30 noncarriers) from 2 large Swedish LQT1 founder populations segregating p.Y111C (22 index families) or p.R518X (13 index families). Each founder pedigree was constructed providing identity by descent (data on parentage) for all essential individuals linking the included cases, resulting in 2 separate pedigrees with a total of 889 individuals (Figure 1).

Among the 184 included cases with available fetal data, 74 were noncarriers and 110 were mutation carriers, all ascertained by molecular genetic testing except for 1 child with clinical JLNS diagnosis, who died suddenly before molecular genetic testing. Among the 110 mutation carriers, 97 were carriers of single LQT1 mutations and 13 were carriers of double LQT1 mutations (including the 1 untested JLNS case). Among fetuses with single LQT1 mutations, the majority was of p.Y111C (n=70) or p.R518X (n=24) genotype, however 3 fetuses, pertaining to a family where both parents were LQT1 mutation carriers (p.R518X and p.A525T), carried the p.A525T mutation. Among the cases with double LQT1 mutations (all from the p.R518X population), 9 had congenital hearing loss (although 1 had residual hearing in 1 ear) and 4 had normal hearing. An overview of the study population, including clinical characteristics stratified by genotype, is presented in Table 1. Sex was equally distributed within each genotype group, that is, noncarriers, single mutation carriers, and double mutation carriers, (females 49%, 48%, and 46% per group), as well as between carriers of specific LQT1 mutations (Y111C females, 49%; R518X females, 50%). There were no differences in gestational age at delivery between the genotype groups (no mutation, 39±2 weeks; single mutation, 39±2 weeks; and double mutations, 39±1 week).

Among the included 110 mutation carrier fetuses from 35 index families, only 3 probands (all double mutation carriers from the p.R518X founder population) were originally identified based on fetal presentation.

Associations Between Intrauterine Heart Rate and Fetal Genotype
There was a mean of 9±3 intrauterine heart rate recordings per fetus (range, 1–18) including a mean of 6±3 recordings during the third trimester and onwards (range, 1–14). When considering all heart rates from week 29 onwards, including heart rates recorded at admission to the delivery ward, pedigree-based association analysis, including all founder population cases (n=184) revealed that mean heart rates were lower per added mutation (no mutation, 142±6 beats per minute (n=74); single mutation, 133±8 beats per minute (n=97); double mutations, 111±6 beats per minute (n=13); P<0.0001). As several factors may affect fetal heart rate during labor, for calculating group means (Table 1) and all subsequent association analyses only cases with at least 1 prelabor recording during the third trimester were included (n=175, 69 noncarriers, 93 single mutation carriers: p.Y111C=70, p.R518X=20, and p.A525T=3, and 13 double mutation carriers, with 5±2, range 1–13, recordings per fetus). Prelabor mean third trimester fetal heart rate (henceforth referred to as fetal heart rate) was lower in mutation carriers (no mutation, 143±5 beats per minute [n=69] versus any mutation, 131±10 beats per minute [n=106]; P<0.0001), and lower per added mutation (no mutation, 143±5 beats per minute [n=69] versus single mutation, 134±8 beats per minute [n=93]; P<0.0001; single mutation, 134±8 beats per minute versus double mutations, 111±6 beats per minute [n=13]; P<0.0001, Table 1; Figure 2A). Also, there was a significant association between fetal heart rate and specific genotype ordered according to predicted KCNQ1 function-loss (no mutation [n=69], p.R518X [n=20], p.Y111C [n=70], compound heterozygous p.R518X/+ [n=9], and homozygous p.R518X/p.R518X [n=3], P<0.0001; Figure 2B). Among single mutation carriers, there was a trend toward lower heart rates in fetuses carrying the dominant-negative p.Y111C mutation (n=70), when compared with fetuses carrying the nonsense mutation p.R518X (n=20), causing haploinsufficiency, although the difference was not statistically significant (133±8 versus 137±6 beats per minute, P=0.057; Table 1; Figure 2B). There was no association between fetal heart rate and sex in the study population as a whole, or when taking fetal genotype into account (P=0.33).

Figure 1. Overview of the relatedness data used in the measured genotype association analysis performed by Sequential Oligogenic Linkage Analysis Routines (SOLAR®; http://www.sfrb.org/solar) and Statistical Analysis for Genetic Epidemiology software (SAGE®; http://darwin.cwru.edu). The estimated pedigrees were constructed based on previously published genealogical and microsatellite data, including all essential individuals (vertical lines) connecting the 184 cases with fetal data back to a common possible founder, for each respective founder population (total n=889, p.Y111C on the left, p.R518X on the right). Horizontal dotted lines represent generations.
Intrauterine Heart Rate and the Effect of Maternal β-Blocker Use

β-blockers were used by the mother in 21/184 pregnancies (11%). Among these pregnancies, fetal genotypes included 7 noncarriers, 12 single mutation carriers, and 2 double mutation carriers. Intrauterine heart rates for fetuses exposed to β-blockers when compared with nonexposed fetuses, stratified by genotype, are presented in Figure 3. Fetal heart rates were significantly lower for fetuses exposed to β-blockers (127±12 versus 137±10 beats per minute; *P* = 0.019), and the finding was consistent within each genotype group (exposed versus nonexposed; noncarriers, 139±5 versus 143±5 beats per minute; single mutation carriers, 124±8 versus 135±6 beats per minute; and double mutation carriers, 105±4 versus 112±6 beats per minute). There was no

Table 1. Clinical Characteristics Stratified by Genotype in 184 Fetuses From 2 Long QT Syndrome Type 1 Founder Populations

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All, n</th>
<th>Male, n (%)</th>
<th>Female, n (%)</th>
<th>Mean HR*, Beats Per Minute</th>
<th>QTc, ms, n (%)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any, all fetuses</td>
<td>184</td>
<td>95 (52)</td>
<td>89 (48)</td>
<td>136±11</td>
<td>488±60, 124 (67)</td>
<td>...</td>
</tr>
<tr>
<td>Noncarriers</td>
<td>74</td>
<td>38 (51)</td>
<td>36 (49)</td>
<td>143±5</td>
<td>421±21, 25 (34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mutation carriers</td>
<td>110</td>
<td>57 (52)</td>
<td>53 (48)</td>
<td>131±10</td>
<td>488±58, 99 (90)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Single mutation</td>
<td>97</td>
<td>50 (52)</td>
<td>47 (48)</td>
<td>134±8</td>
<td>472±35, 86 (89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p.Y111C</td>
<td>70</td>
<td>36 (51)</td>
<td>34 (49)</td>
<td>133±8</td>
<td>475±30, 60 (86)</td>
<td>0.235</td>
</tr>
<tr>
<td>p.R518X</td>
<td>24</td>
<td>12 (50)</td>
<td>12 (50)</td>
<td>137±6</td>
<td>462±41, 23 (96)</td>
<td>0.235</td>
</tr>
<tr>
<td>p.A525T‡</td>
<td>3</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>131±2</td>
<td>490±59, 3 (100)</td>
<td>...</td>
</tr>
<tr>
<td>Double mutations§</td>
<td>13</td>
<td>7 (54)</td>
<td>6 (46)</td>
<td>111±6</td>
<td>595±68, 13 (100)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHZ</td>
<td>9</td>
<td>5 (56)</td>
<td>4 (44)</td>
<td>111±7</td>
<td>584±70</td>
<td>...</td>
</tr>
<tr>
<td>HZ</td>
<td>3</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>110±3</td>
<td>625±80</td>
<td>...</td>
</tr>
<tr>
<td>JNS</td>
<td>9</td>
<td>4 (44)</td>
<td>5 (56)</td>
<td>112±6</td>
<td>573±67</td>
<td>...</td>
</tr>
<tr>
<td>Normal hearing</td>
<td>4</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>110±7</td>
<td>644±46</td>
<td>...</td>
</tr>
</tbody>
</table>

CHZ indicates compound heterozygous (p.R518X/+ other KCNQ1 mutation); HR, heart rate, HZ, homozygous (p.R518X/p.R518X); and JLNS, Jervell and Lange-Nielsen syndrome.

*Mean third trimester HR in beats per minute, presented as mean±SD, calculated from routine prelabor obstetric recordings during weeks 29–41 (excluding 9 cases with delivery ward recordings; 5=noncarriers and 4=p.R518X).

†Between genotypes (no mutation vs any mutation; no mutation vs single mutation; single mutations vs double mutations, and p.Y111C vs p.R518X), calculated by pedigree-based measured genotype association analysis. When no statistical testing was performed this is indicated by (…).

‡Single mutation carriers identified in a compound heterozygous family (A525T/R518X).

§Genotypes included; R518X/R518X=3, A525T/R518X=4, M159sp/R518X=2, R190W/R518X=1, R530W/R518X=1, S349W/R518X=1, and 1 untested (a clinical case with JLNS diagnosis, including congenital deafness, marked QTc prolongation, recurrent syncope, and an aborted cardiac arrest, that died suddenly while playing in the water, before genetic testing).

Figure 2. A. Mean prelabor third trimester fetal heart rates were significantly lower per added KCNQ1 mutation (no mutation, 143±5 beats per minute [n=69] vs single mutation, 134±8 beats per minute [n=93]; *P*<0.0001; single mutation vs double mutations, 111±6 beats per minute [n=13, including 1 Jervell and Lange-Nielsen syndrome case of unascertained genotype]; *P*<0.0001), calculated by pedigree-based measured genotype association analysis. Identical values are stacked. B. There was a significant association between mean third trimester heart rate and specific genotype ordered according to predicted KCNQ1 function-loss (no mutation [n=60], p.R518X [haploinsufficiency,23 n=20], p.Y111C [dominant-negative,‡ n=70], compound heterozygous carriership of p.R518X plus another KCNQ1 mutation [near-complete to complete function-loss, n=9], and homozygous p.R518X carriership [complete function-loss, n=3]; *P*<0.0001), as calculated by pedigree-based measured genotype association analysis. Data from functional studies on p.A525T/−, identified in 3 cases, were not available. Identical values are stacked.
difference in gestational age at delivery between fetuses exposed to β-blockers when compared with nonexposed fetuses (both 39±2 weeks). As expected, there was no clinical correlate for intrauterine β-blocker exposure with respect to later LQTS phenotype (no differences in distribution of QTc means [P=0.38] or symptomatic phenotype [P=0.33] between exposed and nonexposed children). When excluding all fetuses that were exposed to β-blockers during gestation (n=21), the association between genotype (no mutation, single LQT1 mutation, and double LQT1 mutations) and fetal heart rate remained, P<0.0001.

**Intrauterine Heart Rate, Post Partum Heart Rate, and QTc at Diagnosis**

Neonatal heart rates post partum (within the first month of life) were available in 23 cases. In spite of the small sample size, individual neonatal heart rates correlated strongly with fetal heart rate (r=0.7, P<0.001). In the available sample, neonatal heart rates were significantly lower in single mutation carriers when compared with noncarriers (116±10 beats per minute [n=10] versus 137±6 beats per minute [n=5]; P=0.007) as well as lower in double mutation carriers when...
Intrauterine Heart Rate and Cardiac Arrhythmia Risk

Single or repeated recordings of fetal heart rates suggestive of bradyarrhythmias or tachyarrhythmias during prelabor routine checkups (ie, ≤110 or ≥200 beats per minute) were seen in 21 fetuses (11%), all of whom were mutation carriers. Bradycardia ≤110 beats per minute was seen in 7 (7%) of the single LQT1 mutation carriers and 11 (85%) of the double mutation carriers, and tachycardia ≥200 beats per minute was seen in 3 fetuses, 2 single mutation carriers (2%), and 1 double mutation carrier (8%).

Although several of the included mutation carriers have been on β-blockers since infancy, fetal heart rate showed a significant association with later development of arrhythmia symptoms, with lower fetal heart rates in symptomatic mutation carriers when compared with asymptomatic mutation carriers (122±10 versus 137±9 beats per minute; P<0.0001).

Carriage of double mutations was associated with the lowest fetal heart rates (111±6 beats per minute). Among the cases with double mutations and normal hearing, 1 fetus (mean fetal heart rate, 102 beats per minute) experienced intrauterine tachyarrhythmia suspicious of torsade de pointes in utero during gestational week 39 (bursts of fetal heart rate >200 beats per minute alternating with periods of pronounced bradycardia ≤100 beats per minute). During the tachycardic periods, the mother could not feel fetal movements. The fetal arrhythmia disappeared after administration of intravenous potassium to the mother, correcting her serum levels from 3.9 to 4.3 mmol/L. Another case (mean fetal heart rate, 115 beats per minute) with a clinical JLNS diagnosis died suddenly while playing in the water during early childhood. Other postnatal clinical presentations in the double mutations group include frequent syncope, aborted cardiac arrests (4 cases), electrocardiographically verified torsade de pointes/fast ventricular tachycardia or ventricular fibrillation (4 cases), and frequent appropriate implantable cardioverter–defibrillator shocks (3 cases).

Quantitative Genetic Model Explaining Variance in Fetal Heart Rate

Using a maximum likelihood approach exploring the covariates fetal genotype, fetal sex, fetal phenotype, mother’s genotype, and intrauterine β-blocker exposure, 64.5% of the variance in prelabor mean third trimester fetal heart rate was explained in a restricted maximum likelihood model (Table 2). The trait fetal heart rate was found to be highly heritable, with 71% of the variance in fetal heart rate estimated to be attributable to the additive effect of genes (P=0.002). Fetal genotype (no mutation, single LQT1 mutation, or double LQT1 mutations) was the single most important covariate, by itself explaining more than half of the variance in fetal heart rate in single covariate analysis (55%, P=1.0×10⁻²⁸). The final

Table 2. Final Restricted Maximum Likelihood Model Explaining Variance in Third Trimester Fetal Heart Rate (in Beats Per Minute)

<table>
<thead>
<tr>
<th>Covariates*</th>
<th>β</th>
<th>SE</th>
<th>P*Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal genotype (per mutation)</td>
<td>-10.3</td>
<td>0.9</td>
<td>1.0×10⁻²³</td>
</tr>
<tr>
<td>β-Blockers in utero</td>
<td>-6.9</td>
<td>1.6</td>
<td>0.00003</td>
</tr>
<tr>
<td>Mothers’ genotype (carrier)</td>
<td>-2.4</td>
<td>1.2</td>
<td>0.060</td>
</tr>
<tr>
<td>Arrhythmia symptoms</td>
<td>-6.8</td>
<td>1.6</td>
<td>0.00004</td>
</tr>
<tr>
<td>Heritability (SE, 0.2; P=0.002†)</td>
<td>0.710</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual kurtosis</td>
<td>0.600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of variance</td>
<td>0.645</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*indicates β-coefficient (=effect size, in beats per minute).

†Calculated by pedigree-based measured genotype association analysis using Sequential Oligogenic Linkage Analysis Routines (SOLAR), in 175 cases with complete data on all included variables. Nine cases with recordings only from admission to the delivery ward were excluded from analysis.
multivariable model predicted a 10 beats per minute reduction in fetal heart rate per added mutation (beta coefficient, −10.3; SE, 0.9; \( P=1.0\times10^{-23} \); Table 2). The other 2 significantly associated covariates, intrauterine \( \beta \)-blocker exposure, and fetal phenotype, by themselves explained 3% and 24% of total variance in fetal heart rate in single covariate analysis, respectively (albeit with a residual kurtosis >0.8 for the analyses, limiting the reliability of the results). In the final multivariable model (residual kurtosis 0.6, ie, within normal range) exposure to \( \beta \)-blockers in utero or a symptomatic phenotype each predicted a 7 beats per minute reduction in fetal heart rate (beta-coefficient, −6.9; SE, 1.6; \( P=0.00003 \) and beta-coefficient, −6.8; SE, 1.6; \( P=0.00004 \), respectively; Table 2). Performing the analysis using all mean recordings from week 29 onwards (n=184) rendered comparable results (data not shown).

Calculating a quantitative genetic model using SAGE software and the same parameters for primary trait and covariates yielded comparable results, albeit with a lower proportion of variance explained (40%); heritability 76% (\( P=0.00004 \), fetal genotype (estimated effect, −10 beats per minute/mutation; \( P<1.0\times10^{-07} \)), fetal sex (not significant, \( P=0.60 \)), fetal phenotype (estimated effect, −6.8; \( P=0.00002 \)), mothers’ genotype (not significant, \( P=0.06 \)), and intrauterine \( \beta \)-blocker exposure (estimated effect, −6.8; \( P=0.00002 \)).

Fetal Heart Rate as a Marker for LQTS Suspicin: An Example

A fetal heart rate less than the third percentile for gestational age has been previously suggested as a cutoff for LQTS suspicion (~128–134 during the third trimester).\(^{15}\) Correspondingly, in the LQT1 founder populations, a cutoff of \( \leq 133 \) beats per minute (−2 SD of the mean heart rate of the noncarriers, and comparable with that of a relatively large published normal population\(^{15}\)) would initially detect 50 true positives/mutation carriers and 2 false positives/noncarriers (ie, a sensitivity <50% and a specificity >97%, when considering all mutation carriers), including 81% of the symptomatic cases (Figure 6). When considering specific genotypes, the cutoff would detect 100% of double mutation carriers and 100%, 41%, and 25% of p.A525T, p.Y111C, and p.R518X carriers, respectively. Cascade screening of the detected probands would thereafter identify 19 of their carrier siblings, and another 28 relatives carrying their familial mutation, resulting in a total of 97 identified cases (88% of all mutation carriers in the study, and among them 100% of the symptomatic cases).

Discussion

This retrospective study on 184 fetuses from 2 LQT1 founder populations, including 110 mutation carriers, is by far the largest study to date regarding fetal LQTS, and the only one to include a significant number of double mutations. Several novel findings are of clinical relevance. In a multivariable analysis, fetal genotype, fetal phenotype (arrhythmia), and intrauterine exposure to \( \beta \)-blockers were identified as significantly associated to fetal heart rate, explaining 64.5% of its variance. Specifically, we have demonstrated that fetal heart rate is strongly associated with gene carriage status in LQT1, and that fetal heart rate is lower per added \( KCNQ1 \) mutation (−10 beats per minute in the multivariable model). Regarding phenotype, our data reveal that the mean third trimester intrauterine heart rates of LQT1 fetuses correlate with neonatal heart rate, as well as show association with postnatal cardiac phenotype (QTc and arrhythmia). Importantly, although sinus bradycardia is often described as a rather benign manifestation, with a favorable outcome when treated with \( \beta \)-blockers,\(^{13–15,25} \) it is evident from the present study that isolated sinus bradycardia may also be the presenting symptom of a most severe form of LQTS (ie, double mutation carriage),

![Figure 6. Mean third trimester heart rates, stratified by specific genotype, and phenotype indicated by marker shapes (minus sign, no symptoms; ×, arrhythmia symptoms), related to different cutoffs (horizontal lines) representing obstetric standard for bradycardia (<110 beats per minute), reduced fetal heart rate (110–120 beats per minute), and the discussed cut-off for long-QT syndrome (LQTS) suspicion (<133 beats per minute, ie, mean noncarrier heart rate −2 SD). In the LQT1 founder populations, a cutoff ≤133 beats per minute would initially detect <50% of single mutation carriers, 100% of double mutation carriers, and 81% of symptomatic cases. Subsequent cascade screening in the families of the detected cases would identify 88% of all mutation carriers and 100% of symptomatic cases.](http://circep.ahajournals.org/)

![Mean third trimester heart rates, stratified by specific genotype, and phenotype indicated by marker shapes (minus sign, no symptoms; ×, arrhythmia symptoms), related to different cutoffs (horizontal lines) representing obstetric standard for bradycardia (<110 beats per minute), reduced fetal heart rate (110–120 beats per minute), and the discussed cut-off for long-QT syndrome (LQTS) suspicion (<133 beats per minute, ie, mean noncarrier heart rate −2 SD). In the LQT1 founder populations, a cutoff ≤133 beats per minute would initially detect <50% of single mutation carriers, 100% of double mutation carriers, and 81% of symptomatic cases. Subsequent cascade screening in the families of the detected cases would identify 88% of all mutation carriers and 100% of symptomatic cases.](http://circep.ahajournals.org/)
associated with a severe prognosis even when treated with β-blockers.8,26,27 Taken together, these findings strongly suggest that fetal heart rate recordings obtained from routine maternal healthcare may be useful both for early suspicion of LQTS diagnosis, in particular for those fetuses at the highest risk of early life-threatening arrhythmia, and as an early risk stratification tool with implications for postnatal phenotype.

Importantly, although conflicting results about the effect of intrauterine β-blocker exposure on LQTS fetuses have been previously presented,15,28 the present study indicates that intrauterine β-blocker exposure is associated with significant fetal heart rate reduction irrespective of genotype. Thus, although the main results were not altered when excluding exposed fetuses, intrauterine β-blocker exposure is a variable that needs to be taken into consideration when evaluating fetal heart rate in LQTS families.

Genotype–Phenotype Correlations in Fetal LQT1
An association between LQT1 genotype and sinus bradycardia has previously been reported in neonates.12 With regards to fetal LQTS, little genotype-specific data have previously been presented, although several studies report intrauterine bradycardia in LQTS fetuses.13–15,25,26,29 In the largest previous study on fetal LQT2 (genotype ascertained fetuses: 23 LQT1/6 LQT2/6 LQT3), no genotype-specific hypotheses were tested because of the small sample size, however, the LQT1 subgroup was described as having predominantly sinus rhythm because of the small sample size, however, the LQT1 subgroup was described as having predominantly sinus rhythm (defined as fetal heart rate less than the third percentile for gestational age and absence of atrioventricular block or ventricular tachycardia) were reported to be of LQT1 genotype.13

In this present study including 110 mutation carriers from 2 LQT1 founder populations, fetal heart rate manifestations were clearly genotype dependent, ranging from mild in single mutation carriers to pronounced in double mutation carriers. Even within the single-mutations group, carriers of the p.R518X nonsense mutation (associated with a 50% KCNQ1 function-loss in vitro23) presented with a tendency toward milder heart rate reduction and QTc prolongation than carriers of the dominant negative p.Y111C mutation (associated with >75% KCNQ1 function-loss in vitro23). Among the double mutation carriers, all had p.R518X on 1 allele (resulting in a truncated protein product and a subunit that do not assemble into functional ion channels23) and the majority (69%) had a nonidentical missense or splice-site mutation on the other allele, resulting in a near-complete to complete KCNQ1 function-loss. Evidence of some residual KCNQ1 function was seen in 5 compound heterozygous cases (normal hearing in 4 and residual hearing on 1 ear in 1), however, the fetal manifestations were severe throughout the double mutations group, suggesting that a near-complete KCNQ1 function-loss is sufficient to cause the fetal manifestations, and that our findings would be generalizable to the larger group of double-mutation carriers with normal hearing, at least when carrying LQT1 mutations. Although it is still incompletely understood how dysfunction in ion channel subunits encoded by mutant KCNQ1 genes lead to relative fetal bradycardia, our data strongly suggest that there is a dose–response relationship between the level of potassium channel function-loss on the one hand (corresponding to genotype) and the level of fetal heart rate decrease on the other.

Fetal Heart Rate as a Marker for Suspicion of LQTS; Potential and Limitations
Based on this study, it is clear that fetal heart rate recordings obtained from routine maternal healthcare may be useful for predicting both LQT1 mutation burden and disease severity, at least within LQTS populations, with the caveat that maternal β-blocker usage must be taken into account. Importantly, fetal heart rates are already monitored routinely within clinical practices, that is, these data are potentially available without much additional effort or cost. It is also evident from this and previous studies15 that the current obstetric standard for fetal bradycardia (≤110 beats per minute) is not useful with regards to LQTS, and that we need a higher index of suspicion for LQTS in this context. The question remains, however, as to what level of relative fetal heart rate decrease should signal a need for further follow-up (as well as what prenatal or postnatal investigations would be appropriate). As reported in the Results, applying a cutoff corresponding to the previously suggested less than the third percentile for gestational age15 to our LQTS founder populations identified all double-mutation carriers (ie, all cases at highest risk of early life-threatening cardiac events, irrespective of auditory phenotype) and, after taking cascade-screening into account, all symptomatic LQTS cases. Because of the effectiveness of cascade-screening once a proband has been identified, one could advocate for a strategy to primarily find the cases with the most pronounced fetal heart rate decrease, that is, the cases most likely to present as clinical probands, and correspondingly, a somewhat lower cutoff may be more appropriate to limit false positives. Importantly, the predictive value of fetal heart rate in an unselected population remains unclear, and further studies on both normal populations and unselected LQTS populations are needed to further characterize fetal LQTS and optimize clinically appropriate cutoffs for LQTS suspicion, taking into account positive and negative predictive values as well as the psychological burden of false-positive findings. That said, evaluation of heart rates already monitored in the general fetal population has the potential to significantly improve presymptomatic identification of the most severe LQTS cases, constituting a promising step toward preventing sudden cardiac death in the young.

Study Limitations
This study includes families and cases with LQT1 mutations, within 2 founder populations, and therefore the results may not be generalizable to the entire LQTS population. Genotype was ascertained within routine clinical praxis, and unfortunately some probands and the majority of family members were not screened for additional mutations in other LQTS susceptibility genes. Because of being a retrospective study, the availability and quality of data varies, such as the number of available heart rate recordings per fetus, the availability of ECG recordings, and specifically the age at the ECG recordings (neonatal period–adulthood), which limits comparisons as QTc levels are age dependent.90 Moreover, based on available data, atrioventricular conduction (1:1 or 1:2) in utero could not be ascertained, precluding detection of transient or functional atrioventricular block, however, revision of available postpartum ECGs in cases with persistent bradycardia revealed apparent sinus rhythm with 1:1 conduction.
Conclusions
In this study including 184 fetuses from Swedish LQT1 families, third trimester fetal heart rate discriminated between fetal genotypes (no mutation, single mutation, and double mutations) and showed significant association with later LQTS phenotype. In a multivariable analysis, fetal genotype was the major contributor to fetal heart rate variance, together with phenotype (arrhythmia symptoms) and maternal β-blocker usage. This study thus extends the role of fetal heart rate from early diagnosis to a novel risk stratification marker for familial LQTS, and particularly for the identification of fetuses with double mutations, at high risk of early life-threatening arrhythmias.

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Disclosures
None.

References


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