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

Running title: Winbo et al.; Fetal heart rate in KCNQ1 mutation-carriers

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Abstract:

**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 two LQT1 founder populations segregating p.Y111C and p.R518X (74 non-carriers and 110 KCNQ1 mutation-carriers, whereof 13 double mutation-carries). Pedigree-based measured genotype analysis revealed significant associations between fetal heart rate, genotype and phenotype; mean third trimester pre-labor fetal heart rates obtained from obstetric records (gestational week 29-41) were lower per added mutation (no mutation 143±5 bpm, single mutation 134±8 bpm, double mutations 111±6 bpm, p<0.0001), and lower in symptomatic vs. asymptomatic mutation-carriers (122±10 bpm vs. 137±9 bpm, p<0.0001). Strong correlations between fetal heart rate and neonatal heart rate (r=0.700, p<0.001), and postnatal QTc (r=-0.762, p<0.001) were found. In a multivariable model, fetal genotype explained the majority of variance in fetal heart rate (~10 bpm per added mutation, p<1.0 x 10^-23). Arrhythmia symptoms and intrauterine beta-blocker exposure each predicted -7 bpm, p<0.0001.

**Conclusions** - In this study including 184 fetuses from two 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.

**Keywords:** long QT syndrome, fetal, heart rate, genotype, diagnostic method, genotype-phenotype correlations
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 SCNA3 genes, corresponding to LQT1-3. LQTS prevalence has been estimated to ~1:2000, and it has been reported that 5-10% 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 (1:200 000) where carriehship of LQTS mutations has been estimated to be 1:250-1000.

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 (bpm) and a reduced baseline fetal heart rate (110-120 bpm), with the latter two 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 less than 50 mutation-carriers, leading to a paucity of genotype-specific data. Moreover, little is known regarding 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\textsuperscript{16} and p.R518X\textsuperscript{11} mutations, in order to investigate whether fetal heart rate differs between LQT1 genotype groups (including non-carriers, carriers of single LQT1 mutations and carriers of double LQT1 mutations). Furthermore, 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 two large Swedish founder populations, segregating either p.Y111C\textsuperscript{16} (22 index families) or p.R518X\textsuperscript{11} (13 index families), where cascade-screening for \textit{KCNQ1} gene (LQT1) mutations had been previously performed in the clinical setting. The term family here indicates the first ascertained index case/proband in a family without known relations to any other LQTS family, plus all tested family members identified through the process of cascade screening of first-degree relatives.\textsuperscript{11,16}

Using pedigrees of the cascade-screened families, mothers with children of ascertained genotype born from 1980 and onwards were invited to participate in the study (irrespective of maternal genotype). Among the mothers who wished to participate in the study, all pregnancies, including carrier and non-carrier fetuses, were included in the analysis. The time frame (1980-2014) was chosen based on accessibility and quality of clinical maternal health care records. All participants signed an informed consent and the study was approved by the Regional Ethical Committee in Umeå, Umeå University, Sweden.

Genotype ascertainment

As previously described\textsuperscript{11,16}, ascertainment of carriehip status had been performed according to
then clinical praxis, using genomic DNA extracted by a standard salting-out procedure, and genotypes in index cases had been ascertained by denaturing high-performance liquid chromatography (Wave 3500 HT, Transgenomic, Inc, Omaha, Neb) and/or sequencing all coding exons of the KCNQ1 gene (CEQ 8000, Beckman Coulter, Fullerton, CA, USA). In some probands more than one 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, USA).11,16

Clinical data

For each fetus, the following retrospective data were obtained from pedigrees and clinical records including maternal health care records: information regarding the mothers’ genotype and beta-blocker usage, fetal sex, fetal genotype, fetal heart rate per gestational week, gestational age at birth, electrocardiograms (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 bi-monthly 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 health care records, from gestational week 29 and onwards, excluding recordings of tachycardia ≥ 200 bpm. The mean third trimester heart rate was used, as opposed to using data from the entire fetal period as i) routine check-ups are most frequent during this period and ii) fetal heart rates are gestational age dependent and show a gradual decrease as gestation proceeds.15 Recordings of
either fetal bradycardia (defined as fetal heart rate ≤ 110 bpm, obstetric standard) or tachycardia 
(≥200 bpm) at any time during gestation were noted, for each fetus.

The first 12-lead ECG performed postnatally, typically recorded at 50 millimeter per 
second sweep speed, was obtained from medical records and measured manually by one 
observer. QT intervals were measured, preferably in lead II, as a mean of three consecutive QT 
intervals, and corrected for heart rate by Bazett’s formula (QT/√R-R), using the mean of the R-R 
intervals preceding the measured beats. When available, neonatal heart rates (in bpm) 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 mean 
± standard deviation for continuous variables. Pearson correlations were calculated between 
continuous variables/covariates and results presented with correlation coefficient (r) and 
associated p-value. Due to the non-independence among family members in the two included 
founder populations17, analyses of variance for single and combined covariates were performed 
using pedigree-based Measured Genotype Association Analysis in Sequential Oligogenic 
Linkage Analysis Routines software (SOLAR)18 (http://www.sfbr.org/solar) and findings 
validated using Statistical Analysis for Genetic Epidemiology software (SAGE)19 
(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.11,16,20 The measured
genotype approach, 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 or double mutations=2), fetal sex (female=1, male=2), fetal phenotype (no arrhythmia symptoms=0, syncope and/or intrauterine arrhythmia=1), mothers’ genotype (non-carrier=0, mutation-carrier=1) and intrauterine beta-blocker exposure (no exposure=0, exposure to beta-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 heritability (H2r: 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 beta 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 two-tailed p value of <0.05 was considered statistically significant. Figures were constructed using Inkskape (Open Source software) and IBM SPSS Statistics 19.

Results

Study population

The study included 184 pregnancies in 87 mothers (57 carriers of single LQT1 mutations and 30 non-carriers) from two 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 two separate pedigrees with a total of 889 individuals (Figure 1).

Among the 184 included cases with available fetal data, 74 were non-carriers and 110 were mutation-carriers, all ascertained by molecular genetics testing except for one child with clinical JLNS diagnosis, who died suddenly prior to molecular genetics testing. Among the 110 mutation-carriers, 97 were carriers of single LQT1 mutations and 13 were carriers of double LQT1 mutations (including the one 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 one had residual hearing in one 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, i.e. non-carriers, 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 bpm (n=74), single mutation 133±8 bpm (n=97), double mutations 111±6 bpm (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 one pre-labor recording during the third trimester were included (n=175, 69 non-carriers, 93 single mutation-carriers: p.Y111C=70, p.R518X=20, p.A525T=3) and 13 double mutation-carriers, with 5±2, range 1-13, recordings per fetus. Pre-labor mean third trimester fetal heart rate (henceforth referred to as fetal heart rate) was lower in mutation-carriers (no mutation 143±5 bpm (n=69) vs. any mutation 131±10 bpm (n=106), p<0.0001), and lower per added mutation (no mutation 143±5 bpm (n=69) vs. single mutation 134±8 bpm (n=93), p<0.0001; single mutation 134±8 bpm vs. double mutations 111±6 bpm (n=13), p<0.0001, Table 1 and 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\textsuperscript{23} (n=20), p.Y111C\textsuperscript{24} (n=70), compound heterozygous p.R518X/+ (n=9) and homozygous pR518X/p.R518X (n=3), p<0.0001, Figure 2B). Among single mutation-carriers there was a trend towards lower heart rates in fetuses carrying the dominant-negative p.Y111C mutation (n=70), as compared to fetuses carrying the nonsense mutation p.R518X (n=20), causing haploinsufficiency, although the difference was not statistically significant (133±8 bpm vs.137±6 bpm, p=0.057, Table 1 and 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)
Intrauterine heart rate and the effect of maternal beta-blocker use

Beta-blockers were used by the mother in 21/184 pregnancies (11%). Among these pregnancies, fetal genotypes included 7 non-carriers, 12 single mutation-carriers and 2 double mutation-carriers. Intrauterine heart rates for fetuses exposed to beta-blockers as compared to non-exposed fetuses, stratified by genotype, are presented in Figure 3. Fetal heart rates were significantly lower for fetuses exposed to beta-blockers (127±12 bpm vs. 137±10 bpm, p=0.019), and the finding was consistent within each genotype group (exposed vs non-exposed; non-carriers 139±5 bpm vs. 143±5 bpm, single mutation-carriers 124±8 bpm vs. 135±6 bpm and double mutation-carriers 105±4 bpm vs. 112±6 bpm). There was no difference in gestational age at delivery between fetuses exposed to beta-blockers as compared to non-exposed fetuses (both 39±2 weeks). As expected, there was no clinical correlate for intrauterine beta-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 non-exposed children). When excluding all fetuses that were exposed to beta-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 as compared to non-carriers (116±10 bpm (n=10) vs. 137±6 bpm (n=5), p=0.007) as well as lower in double mutation-carriers as compared to single mutation-carriers (105±7 bpm (n=8) vs. 116±10 bpm (n=10), p=0.003).
Postnatal ECGs were available in 124 cases, whereof 25 non-carriers, 86 single mutation-carriers and 13 double mutation-carriers (Table 1). As expected, QTc averages from available postnatal ECGs were longer per added mutation (non-carriers 421±21 ms vs single mutation-carriers 472±35 ms, p<0.0001, single mutation-carriers 472±35 ms vs. double mutation-carriers 595±68 ms, p<0.0001, Table 1). Also as expected, QTc measurements were longer in cases who presented with intrauterine or postnatal tachyarrhythmia (531±71 ms vs. 465±50 ms, p=0.0001).

Importantly, there was a strong inverse correlation (r=-0.7, p<0.001) between fetal heart rate and QTc recorded at diagnosis (ranging from first day of life – adulthood) in all mutation-carriers. In effect, the lower the heart rate in utero, the longer the QTc at diagnosis, also when taking genotype into account (Figure 4). When excluding the double mutation-carriers from the analysis, the inverse correlation weakened, albeit remained significant (r=-0.4, p<0.001).

Although single mutation-carriers presented with a wide range of fetal heart rates (102-147 bpm), fetal heart rate correlated with mutation carriage and QTc also within founder population nuclear families, as exemplified in the presented pedigrees, including all p.Y111C sibships with both non-carriers and single mutation-carriers, and complete data on fetal heart rate and postnatal QTc (Figure 5).

**Intrauterine heart rate and cardiac arrhythmia risk**

Single or repeated recordings of fetal heart rates suggestive of brady- and/or tachyarrhythmias during pre-labor routine check-ups (i.e ≤110 bpm or ≥200 bpm) were seen in 21 fetuses (11%), all of whom were mutation-carriers. Bradycardia ≤110 bpm was seen in 7 (7%) of the single LQT1 mutation carriers and 11 (85%) of the double mutation-carriers, and tachycardia ≥200 bpm was seen in three fetuses, two single mutation-carriers (2%) and one double mutation-carrier (8%).
Although several of the included mutation-carriers have been on beta-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 as compared to asymptomatic mutation-carriers (122±10 bpm vs. 137±9 bpm, p<0.0001).

Carriage of double mutations was associated with the lowest fetal heart rates (111±6 bpm). Among the cases with double mutations and normal hearing, one fetus (mean fetal heart rate 102 bpm) experienced intrauterine tachyarrhythmia suspicious of Torsade de Pointes in utero during gestational week 39 (bursts of fetal heart rate >200 bpm alternating with periods of pronounced bradycardia ~100 bpm). 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 mmol/l to 4.3 mmol/l. Another case (mean fetal heart rate 115 bpm) 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 ICD 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 beta-blocker exposure, 64.5% of the variance in pre-labor 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 x 10^{-28}). The final multivariable model predicted a 10 bpm reduction in fetal heart rate per added mutation (beta coefficient -10.3, standard error 0.9, p=1.0 x 10^{-23}, Table 2). The other two 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, i.e. within normal range) exposure to beta-blockers in utero or a symptomatic phenotype each predicted a 7 bpm reduction in fetal heart rate (beta coefficient -6.9, standard error 1.6, p=0.00003 and beta coefficient -6.8, standard error 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=0.0004), fetal genotype (estimated effect -10 bpm/mutation, p < 1.0 x 10^{-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 suspicion: an example**

A fetal heart rate less than the third percentile for gestational age has been previously suggested as a cut-off for LQTS suspicion (~128-134 during the third trimester). Correspondingly, in the LQT1 founder populations, a cut-off of ≤133 bpm (~2 standard deviations of the mean heart rate of the non-carriers, and comparable to that of a relatively large published normal population)
would initially detect 50 true positives/mutation-carriers and 2 false positives/non-carriers (i.e. 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 cut-off 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 two 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 bpm 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 shows association with postnatal cardiac phenotype (QTc and arrhythmia). Importantly, while sinus bradycardia is often described as a rather benign manifestation, with a favorable outcome when treated with beta-blockers, it is evident from the present study that isolated sinus bradycardia may also be the presenting symptom of a most severe form of LQTS (i.e. double mutation carriage), associated with a severe prognosis even when treated with
beta-blockers.8, 26, 27 Taken together, these findings strongly suggest that fetal heart rate recordings obtained from routine maternal health care 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, while conflicting results regarding the effect of intrauterine beta-blocker exposure on LQTS fetuses have been previously presented,15, 28 the present study indicates that intrauterine beta-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 beta-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.25 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, 28, 29

In the largest previous study on fetal LQTS (genotype ascertained fetuses: 23 LQT1/6 LQT2/6 LQT3), no genotype-specific hypotheses were tested due to the small sample size, however the LQT1 subgroup was described as having predominantly sinus rhythm and a mild bradycardia.15 In a later study on the same cohort, 21/32 fetal cases presenting with isolated sinus bradycardia (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 the present study including 110 mutation-carriers from two 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 vitro\textsuperscript{23}) presented with a tendency towards milder heart rate reduction and QTc prolongation than carriers of the dominant negative p.Y111C mutation (associated with >75% KCNQ1 function-loss in vitro\textsuperscript{24}). Among the double mutation-carriers, all had p.R518X on one allele (resulting in a truncated protein product and a subunit that do not assemble into functional ion channels\textsuperscript{23}) and the majority (69%) had a non-identical 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 one ear in one), 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. While 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 the present study, it is clear that fetal heart rate recordings obtained from routine maternal health care may be useful for predicting both LQT1 mutation burden and disease severity, at least within LQTS populations- with the caveat that maternal beta-blocker usage must be taken into account. Importantly, fetal heart rates are already monitored routinely within clinical practices, i.e. these data are potentially available without much additional effort or cost. It is also evident from this and previous studies\textsuperscript{15} that the current obstetric standard for fetal
bradycardia (≤110 bpm) 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 pre- and/or postnatal investigations would be appropriate). As reported in the results section, applying a cut-off corresponding to the previously suggested less than the third percentile for gestational age\textsuperscript{15} to our LQTS founder populations identified all double-mutation carriers (i.e. 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. Due to 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, i.e. the cases most likely to present as clinical probands, and correspondingly, a somewhat lower cut-off 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 cut-offs 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 pre-symptomatic identification of the most severe LQTS cases, constituting a promising step towards preventing sudden cardiac death in the young.

**Study limitations**

This study includes families and cases with LQT1 mutations, within two 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. Due to 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. 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.

Conclusion
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 beta-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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Conflict of Interest Disclosures: None.
References:


Table 1: Clinical characteristics stratified by genotype in 184 fetuses from two LQT1 founder populations

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<td>Any, all fetuses</td>
<td>184</td>
<td>95</td>
<td>89</td>
<td>136±11</td>
<td>…</td>
<td>488±60</td>
<td>124 (67)</td>
</tr>
<tr>
<td>Non-carriers</td>
<td>74</td>
<td>38</td>
<td>36</td>
<td>143±5</td>
<td>&lt;0.0001</td>
<td>421±21</td>
<td>25 (34)</td>
</tr>
<tr>
<td>Mutation-carriers</td>
<td>110</td>
<td>57</td>
<td>53</td>
<td>131±10</td>
<td>&lt;0.0001</td>
<td>488±58</td>
<td>99 (90)</td>
</tr>
<tr>
<td>Single-mutation</td>
<td>97</td>
<td>50</td>
<td>47</td>
<td>134±8</td>
<td>&lt;0.0001</td>
<td>472±35</td>
<td>86 (89)</td>
</tr>
<tr>
<td>p.Y111C</td>
<td>70</td>
<td>36</td>
<td>34</td>
<td>133±8</td>
<td>=0.057</td>
<td>475±30</td>
<td>60 (86)</td>
</tr>
<tr>
<td>p.R518X</td>
<td>24</td>
<td>12</td>
<td>12</td>
<td>137±6</td>
<td>=0.057</td>
<td>462±41</td>
<td>23 (96)</td>
</tr>
<tr>
<td>p.A525T‡</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>131±2</td>
<td></td>
<td>490±59</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Double mutations§</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>111±6</td>
<td>&lt;0.0001</td>
<td>595±68</td>
<td>13 (100)</td>
</tr>
<tr>
<td>CHZ</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>111±7</td>
<td></td>
<td>584±70</td>
<td></td>
</tr>
<tr>
<td>HZ</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>110±3</td>
<td></td>
<td>625±80</td>
<td></td>
</tr>
<tr>
<td>JLNS</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>112±6</td>
<td></td>
<td>573±67</td>
<td></td>
</tr>
<tr>
<td>Normal hearing</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>110±7</td>
<td></td>
<td>644±46</td>
<td></td>
</tr>
</tbody>
</table>

HR- heart rate, bpm- beats per minute, ms- milliseconds, CHZ- compound heterozygous (p.R518X/ +other KCNQ1 mutation), HZ- homozygous (p.R518X/p.R518X)
* Mean third trimester heart rate in beats per minute, presented as mean± standard deviation, calculated from routine pre-labor obstetric recordings during week 29-41 (excluding 9 cases with delivery ward recordings; 5=non-carriers, 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 one 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, prior to genetics testing).
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>Beta</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 x 10^{-23}</td>
</tr>
<tr>
<td>Beta-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></td>
<td></td>
<td>0.710</td>
</tr>
<tr>
<td>Residual Kurtosis</td>
<td></td>
<td></td>
<td>0.600</td>
</tr>
<tr>
<td>Proportion of variance</td>
<td></td>
<td></td>
<td>0.645</td>
</tr>
</tbody>
</table>

Beta: beta coefficient (effect size, in beats per minute), SE: standard error
*Inclusion criteria in final model p<0.1. Fetal sex (p=0.628) was excluded. QTc was not tested for inclusion (available in 69% only).
†Calculated by pedigree-based measured genotype association analysis using 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.

Figure Legends:

Figure 1: Overview of the relatedness data used in the measured genotype association analysis performed by SOLAR [18] (http://www.sfbr.org/solar) and SAGE [19] (http://darwin.cwru.edu). The estimated pedigrees were constructed based on previously published genealogical and microsatellite data, [11,16,20] 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.

**Figure 2:** (A) Mean pre-labor third trimester fetal heart rates were significantly lower per added KCNQ1 mutation (no mutation 143±5 bpm (n=69) vs. single mutation 134±8 bpm (n=93), p<0.0001; single mutation vs. double mutations 111±6 bpm (n=13, including one JLNS 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 (haploinsufficiency23, n=20), p.Y111C (dominant-negative24, 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.

**Figure 3:** Mean third trimester heart rates, stratified by specific genotype, with intrauterine exposure to beta-blockers indicated by marker shapes (minus sign- no exposure, x- beta-blockers in utero). Mean third trimester heart rates were lower in exposed fetuses (127±12 bpm vs. 137±10 bpm, p=0.019, calculated by pedigree-based measured genotype association analysis).
**Figure 4:** A significant inverse correlation ($R^2$ Linear= 0.452) was seen between genotype (no mutation, single mutation and double mutations), third trimester fetal heart rate in beats per minute (bpm) and QTc from available postnatal electrocardiograms (n=124) in two LQT1 founder populations. (minus sign- no mutation, circle- single mutation, plus sign- double mutations)

**Figure 5:** Pedigrees illustrating relationship between mutation-carriage, mean third trimester heart rate and post natal QTc in p.Y111C families including sibling-ships of both non-carrier and single mutation-carrier genotype, and complete data for all parameters (heart rate in beats per minute, on the first line directly below each fetus, and QTc in milliseconds on the second line).

None of the fetuses (n=30) were exposed to beta-blockers in utero. Filled symbols- mutation-carrier, squares- males, circles- females, white border- symptomatic phenotype.

**Figure 6:** Mean third trimester heart rates, stratified by specific genotype, and phenotype indicated by marker shapes (minus sign- no symptoms, x- arrhythmia symptoms), related to different cut-offs (horizontal lines) representing obstetric standard for bradycardia (<110 bpm), reduced fetal heart rate (110-120 bpm) and the discussed cut-off for LQTS suspicion (≤133 bpm i.e. mean non-carrier heart rate -2 standard deviations). In the LQT1 founder populations a cut-off ≤133 bpm 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.
Mean third trimester heart rate (bpm)

Genotype according to predicted KCNQ1 function-loss

- no mutation
- R518X
- Y111C
- R518X/+ 
- R518X/R518X
Third Trimester Fetal Heart Rate Predicts Phenotype and Mutation Burden in the Type 1 Long QT Syndrome
Annika Winbo, Inger Fosdal, Maria Lindh, Ulla-Britt Diamant, Johan Persson, Göran Wettrell and Annika Rydberg

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