Mod Rheumatol
DOI 10.1007/s10165-012-0785-8

RAPID COMMUNICATION

The crucial roles of IFN-γ in the development of M3 muscarinic acetylcholine receptor induced Sjögren's syndrome-like sialadenitis

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Received: 20 July 2012 / Accepted: 10 October 2012

Keywords Sjögren's syndrome · M3 muscarinic acetylcholine receptor · IFN-γ · Apoptosis · Sialadenitis

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by infiltration of lymphocytes into lacrimal and salivary glands, and clinically by dry eyes and dry mouth. Auto-antigens recognized by T cells infiltrating the salivary glands of patients with SS have been analyzed, and several candidate auto-antigens such as M3 muscarinic acetylcholine receptor (M3R) have been identified. The presence and specificity of anti-M3R antibodies in patients with SS have been examined [1–3]. We also reported the presence of IFN-γ-producing M3R-reactive $CD4^+$ T cells in 40 % of SS patients with SS [4]. Several studies also detected high levels of IFN-γ in the salivary glands of SS patients, and then enhanced activity of T cells, B cells, and macrophages, resulting in the destruction and dysfunction of tissue glands [5, 6]. In contrast, IL-17-producing T cells were also found in salivary glands from patients with SS [7].

Our previous study showed that M3R-reactive T cells were involved in the pathogenesis of sialadenitis using M3R-induced sialadenitis (MIS) mice, which are thought to be model mice for SS. In MIS mice, $CD3^+$ T cells were essential for the generation of sialadenitis. Moreover, both IFN-γ and IL-17 were produced by M3R-reactive T cells and were detected in salivary glands, whereas neither IFN-γ nor IL-17 was detected in the sera [8]. However, we have no evidence that the cytokines INF-γ and/or IL-17 are important in the development of sialadenitis. In the present study, to address the question of whether IFN-γ is important in the development of sialadenitis, we generated $M3R^{-/-} \times IFN\text{-}\gamma^{-/-}$ mice, immunized with M3R peptides, and transferred their splenic cells to $Rag\text{-}1^{-/-}$ mice.

Histological findings showed that sialadenitis was more severe in $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ than that in $M3R^{+/+} \rightarrow Rag1^{-/-}$ mice, but milder than that in $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice (Fig. 1a). Quantitative analysis using histological scores indicated that mononuclear cell infiltration was significantly increased in $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice compared with that in $M3R^{+/+} \rightarrow Rag1^{-/-}$ mice ($P < 0.05$), but significantly decreased compared with that in $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice ($P < 0.05$) (Fig. 1b). These observations support the notion that IFN-γ might play a crucial role in the generation of SS-like sialadenitis. The absence of IFN-γ- and presence of IL-17-producing cells in the salivary glands of $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice were verified by immunohistochemical staining (Fig. 1c). IL-17-producing cells in inflammatory lesions were identified in both $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ and $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice. IFN-γ and IL-17 were not detected in sera from $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice, nor in $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice (data not shown). In $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice, the expression of IL-17 was also observed in salivary glands, as was IFN-γ expression. As we have no direct evidence in support of a pathogenic role of IL-17 in MIS, further studies using $M3R^{-/-} \times IL\text{-}17^{-/-}$ mice will be necessary to clarify the function of IL-17-producing M3R-reactive T cells.

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Published online: 26 October 2012

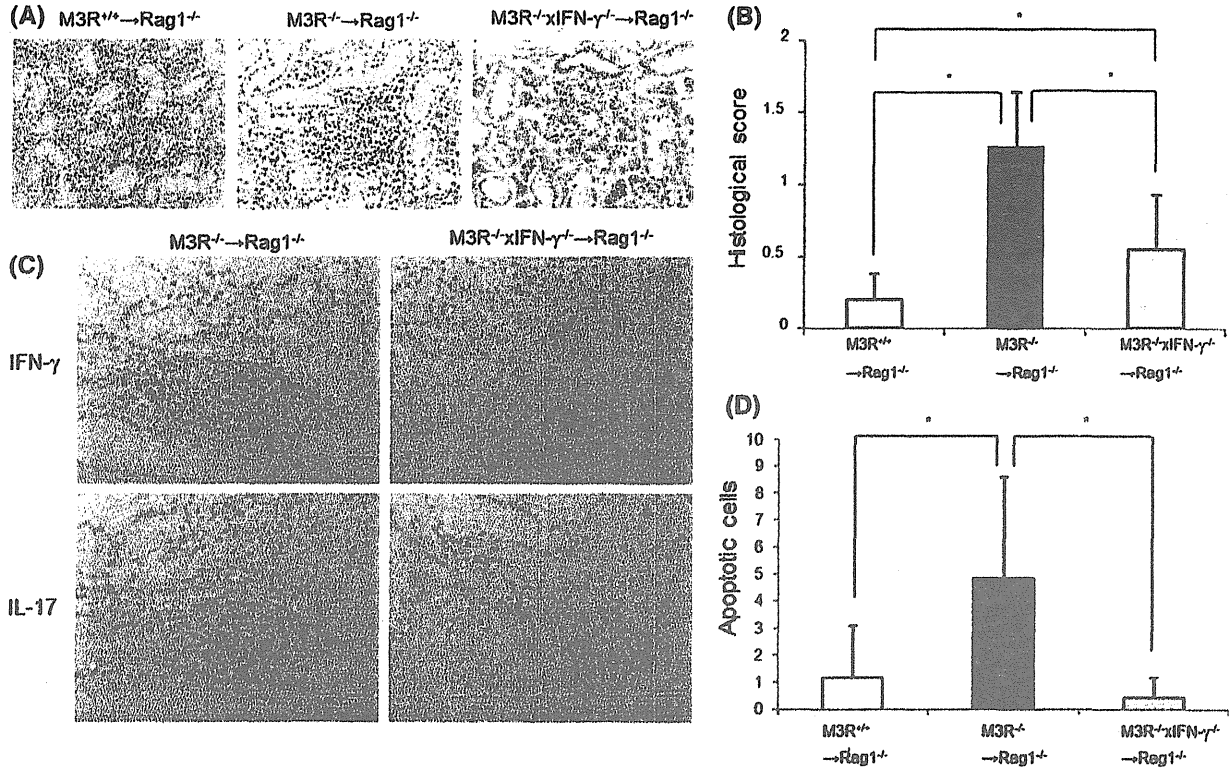

Fig. 1 MIS was reduced in $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice. **a** Salivary glands isolated from $Rag1^{-/-}$ mice at day 45 after inoculation of splenocytes from $M3R^{+/+}$, $M3R^{-/-}$, and $IFN\text{-}\gamma^{-/-}$/$M3R^{-/-}$ mice immunized with M3R peptides encoding the extracellular domains of M3R on days 0 and 10. On the day of immunization, 500 ng of pertussis toxin were injected intraperitoneally. Ten days after booster immunization, the spleens were isolated and transferred into $Rag1^{-/-}$ mice. The salivary glands from $M3R^{+/+} \rightarrow Rag1^{-/-}$, $M3R^{-/-} \rightarrow Rag1^{-/-}$, and $M3R^{-/-}xIFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice were sliced into 4-μm-thick sections, and each section was stained with Mayer's hematoxylin and eosin (H&E). The original magnification was ×100. Representative images of 3–5 mice from each group. **b** The infiltrating cells in salivary glands from $M3R^{+/+} \rightarrow Rag1^{-/-}$, $M3R^{-/-} \rightarrow Rag1^{-/-}$ and $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice were estimated by histological score. The mean + SD are shown. $^*P < 0.05$ (Student's *t* test). **c** Immunohistochemical analysis of IFN-γ and IL-17 in salivary glands of $M3R^{-/-} \rightarrow Rag1^{-/-}$ and $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice. The stained sections were counterstained with H&E, and were observed at 30× the original magnification. Representative images of three mice from each group **d** Apoptotic cells in salivary glands from $M3R^{+/+} \rightarrow Rag1^{-/-}$, $M3R^{+/+} \rightarrow Rag1^{-/-}$, and $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice were measured by TUNEL staining and described as the number of cells in the objective area of 4 mm^2. Data were analyzed in three fields per section. The mean + SD are shown. $^*P < 0.05$ (Student's *t* test)

Several studies have shown that IFN-γ is able to stimulate various cells to express Fas, which triggers apoptosis when stimulated by FasL for its ligand [9, 10]. In our study, the number of apoptotic cells in salivary glands in $M3R^{-/-} \times IFN\text{-}\gamma^{-/-} \rightarrow Rag1^{-/-}$ mice was significantly reduced compared to the number of apoptotic cells in salivary glands in $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice ($P < 0.05$), although apoptotic cells were enhanced in $M3R^{-/-} \rightarrow Rag1^{-/-}$ mice in comparison with those in $M3R^{+/+} \rightarrow Rag1^{-/-}$ mice (Fig. 1c). These findings indicate that IFN-γ plays an important role in the apoptosis of epithelial cells and mononuclear cells in salivary glands in MIS mice.

In conclusion, our observations support the notion that IFN-γ-producing M3R-reactive T cells play a crucial role in the generation of SS-like sialadenitis via the induction of apoptosis. Hence, these results suggest the possibility that IFN-γ-targeting therapy could be used to regulate autoimmune sialadenitis in patients with SS.

Acknowledgments This work was supported by Health and Labour Science Research Grants (2012) for research on intractable diseases, The Clinical Study Group for Autoimmune Diseases.

Conflict of interest None.

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Mod Rheumatol
DOI 10.1007/s10165-012-0704-z

ORIGINAL ARTICLE

The predictive value of anti-SS-A antibodies titration in pregnant women with fetal congenital heart block

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Received: 7 March 2012 / Accepted: 13 June 2012

Abstract
Objective Fetal congenital complete heart block (CHB) is irreversible and is associated with significant mortality and morbidity. Anti-SS-A antibodies in the maternal sera are involved in its pathogenesis; however, the predictive value of the antibody titer and its role in prediction of this complication are controversial. The aim of this study was to determine the predictive value of maternal anti-SS-A antibodies on the development of fetal CHB.
Methods A retrospective chart review was performed for 189 cases of positive anti-SS-A antibodies determined by the double immunodiffusion (DID) method, and included 17 patients that developed fetal CHB. The relationship between the appearance of CHB and the anti-SS-A antibodies titer was examined.
Results An anti-SS-A antibodies titer of 1:32 or higher was identified by analyzing the receiver-operating characteristics (area under curve 0.72) curve. An anti-SS-A antibodies titer of 32 or more times greater than the upper limit by DID was a risk factor for fetal CHB (odds ratio 27.77, 95 % confidence interval (CI) 1.91–21.02, $P < 0.05$) in the multivariate analysis. Among 107 cases of anti-SS-A antibodies titers of 1:32 or higher, 65 patients (60.7 %) were treated with oral steroids. Of these, four patients had CHB (6.2 %). This rate of CHB was significantly lower ($P < 0.01$) than the rate in patients not treated with steroids.
Conclusion An anti-SS-A antibodies titer of 1:32 or higher in the maternal sera by DID was an independent risk factor for fetal CHB. In these patients, either antenatally administered prednisolone or betamethasone, was associated with a lower risk of fetal CHB.

Keywords Connective tissue diseases · Anti-SS-A antibodies · Pregnancy

Electronic supplementary material The online version of this article (doi:10.1007/s10165-012-0704-z) contains supplementary material, which is available to authorized users.

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Introduction

Neonatal lupus erythematosus (NLE) is, in many cases a passively acquired autoimmune syndrome in which pathogenic autoantibodies (anti-SS-A antibodies) are transmitted from a mother to her fetus through the placenta. NLE is frequently associated with the presence of anti-SS-A antibodies in the mother [1].

Among the major clinical manifestations in infants with NLE, complete congenital heart block (CHB) is irreversible and requires the early implantation of a permanent

Published online: 04 July 2012

pacemaker. In contrast, the non-cardiac manifestations are transient, resolving by 6 months of age without specific treatment [2]. CHB carries a significant mortality and morbidity including permanent pacing before adulthood [3]. The prevalence of CHB in children from women previously known to have anti-SS-A antibodies is ~1–2 % [4]. Thus, the prevention of CHB is an important issue in the management for pregnant women who test positive for anti-SS-A antibodies.

Several recent reports address the therapeutic approaches for fetal heart block. Findings of the PR interval and dexamethasone evaluation (PRIDE) study suggest that the PR interval should be measured regularly in fetuses at risk of heart block [5, 6]. Regular assessment of the fetal PR interval, however, may prove to be unduly burdensome for patients and physicians [7].

Transplacental steroid therapy has been proposed as a means of preventing CHB. Betamethasones are administered if the mother is positive for anti-SS-A antibodies, or has a history of a previous child with CHB. The efficacy of this, as well as that of dexamethasone, however, remains controversial [8]. Additionally, there is concern regarding the adverse neurodevelopmental effects of prenatal steroid exposure, thus a careful neurological assessment of fetuses treated with steroids is required [9].

The empiric treatment of all pregnant patients who test positive for anti-SS-A antibodies may subject an excessive number of fetuses to the detrimental effects of steroids. Therefore, a need exists for a means by which to identify the subset of fetuses at high risk of CHB who may benefit from steroids. While the 52 kD SS-A/Ro or 60 kD SS-A/Ro antibodies are clearly associated with CHB, other factors affect susceptibility [8]. For example, a prior history of CHB increases the risk ninefold, to 19 %, in subsequent pregnancies [4, 10]. Therefore, we reviewed the clinical courses of the patients in this study to identify other contributory factors, in addition to the anti-SS-A antibodies titer, that predicted the development of CHB.

Methods

Patients entered in this retrospective study were followed at one of five Japanese tertiary perinatal centers, including Kyushu University Hospital, Juntendo University, the University of Tsukuba, Osaka Medical Center and Research Institute for Maternal and Child, National Center for Child Health and Development, between 1996 and 2009. A total of 214 pregnant women with SS-A antibodies were enrolled in this study, and in 189 cases, anti-SS-A antibodies were titered by DID (double immune—diffusion) using commercially available kits (ENA-2 test, MBL, Nagoya, Japan or SRL, TFB, Tokyo, Japan). The correlation between these kits was verified by the supplier (personal communication).

Serum samples from 189 patients that were positive on an immunofluorescent screening test using HEp-2 cells were analyzed for anti-SS-A antibodies by DID in each laboratory using its current in-house methodology. The protocol numbers were: 21-71, 21-114, 22-610, 364 and 436 for Kyushu University Hospital, Juntendo University, University of Tsukuba, Osaka Medical Center and Research Institute for Maternal and Child, National Center for Child Health and Development, respectively. The study protocol opened for enrollment at each institution following approval by the ethics committee at each site.

The patients were divided into two groups based on whether the fetus developed CHB. A retrospective chart review was then performed to record maternal demographic characteristics, such as maternal age, parity, gestational week at delivery, frequency of premature delivery, deviation from standard birth weight, APGAR score (low APGAR at 5 min <7), antibody titer by DID, signs and symptoms of maternal autoimmune disease, and medications taken before and during the pregnancy. The deviation from the standard birth weight, a widely accepted method for evaluating the fetal growth, was calculated using the following formula: [(Mean weight at corresponding gestational week)-(actual BW)]/(Standard deviation at the corresponding gestational week) [11, 12].

Mean birth weight and the standard deviation at certain gestational ages were calculated using a formula derived from normal values for the Japanese population.

Multiple logistic regression and a receiver-operating characteristics (ROC) curve for levels of anti-SS-A antibodies by DID in the prediction of fetal CHB were calculated using EXCELL Tokei 2010—in Japanese (Shakai Joho Service, Tokyo, Japan). Statistical analysis was performed using the Mann-Whitney test, Chi-square test, and the unpaired t test programmed in GraphPad Prism® (GraphPad Software, Inc., CA). A P value of <0.05 was considered significant.

Results

Clinical profile

Mean age, parity, gestational week at delivery, frequency of premature delivery, birth weight, deviation from the standard birth weight, APGAR score at 5 min, cases with NLE, cases with CHB, in which the elevated anti-SS-A antibodies were demonstrated subsequent to the development of fetal CHB, signs and symptoms of maternal autoimmune disease, diagnosis of maternal autoimmune disease, and medications taken before and during the pregnancy are shown in Table 1.

Table 1 Clinical profiles and comparison of outcomes between cases with or without fetal CHB

	Cases with CHB (n = 17)	Cases without CHB (n = 172)	P values
Age (years)*	30.2 (23.5 to 36.3)	33 (22 to 43.1)	**<0.05**
Gravidity*	0 (0 to 1)	0 (0 to 2)	0.94
Gestational week at delivery*	37 (32 to 39)	38 (29 to 41)	**<0.01**
Premature birth**	5 (29.4 %)	37 (21.5 %)	0.54
Birth weight (g)*	2300 (1172 to 3028)	2584 (948 to 3978)	0.05
Deviation from standard birth weight (SD)*	0.7 (−3.7 to 2.4)	−0.8 (−3.9 to 4.4)	0.90
Apgar score (5 min)*	8 (7–9)	9 (0 to 10)	**<0.01**
Previous child with CHB**	0 (0.0 %)	10 (5.8 %)	0.60
Anti-SS-A titer of 1:32 or higher	15 (88.2 %)	93 (54.1 %)	**<0.01**
Signs and symptoms maternal autoimmune disease**	8 (47.1 %)	131 (76.2 %)	<0.01
Diagnosis of maternal autoimmune disease**	8 (47.0 %)	137*** (79.6 %)	<0.01
Medications taken before and during	4 (23.5 %)	120 (69.8 %)	**<0.01**

Bold values indicate statistically significant

* Mean (range),** number of cases (1 %), + except for cases receiving medication after finding out fetal CHB, *CHB* congenital heart block, *NLE* neonatal lupus syndrome, *** includes seven asymptomatic patients diagnosed by chance, *DID* double immunodiffusion

Data are expressed as either the * median (range) or ** number of cases (%). The p values for the comparison between cases with fetal CHB versus cases without fetal CHB were calculated using either * Mann–Whitney test, ** Chi-square test (GraphPad Prism 5®, GraphPad Software, Inc., CA, USA). P values <0.05 were considered significant

Maternal autoimmune disease diagnoses and clinical details are presented in Table 2.

Predicting values (Fig. 1)

One hundred eighty-nine cases were available for evaluation with a receiver-operating characteristics (ROC) curve of the level of anti-SS-A antibodies (DID) and fetal CHB (morbidity). Based on the ROC curve at a cut-off point of 1:32 for the anti-SS-A antibodies titer, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 87.0, 53.0, 17.1, and 97.4 %, respectively, for predicting cases at high risk for CHB with an area under the curve (AUC) of 0.72 (Fig. 1).

Univariate analysis (Table 1)

A univariate analysis was performed analyzing the relationship between CHB and either maternal age, parity, gestational week at delivery, frequency of premature delivery, APGAR score at 5 min, and an anti-SS-A antibodies titer of 1:32 or higher, classification based on the ROC curve, presence of signs and symptoms of maternal autoimmune disease, diagnosis of maternal autoimmune disease, and medications taken before and during the pregnancy (Table 1).

Maternal age, gestational week at delivery, APGAR score at 5 min, an anti-SS-A antibodies titer of 1:32 or higher, presence of signs and symptoms of maternal autoimmune disease, diagnosis of maternal autoimmune disease, and medications taken before and during the pregnancy showed significant correlations.

Table 2 Clinical diagnosis of outcome between cases with or without fetal CHB

	Cases with CHB (n = 17)	Cases without CHB (n = 172)
Diagnosis of maternal autoimmune disease	8 (47.0 %)	137 (79.6 %)
Sjs	4	46
SLE	1	35
MCTD	0	4
APS	0	1
RA	1	9
Sjs/SLE	0	18
Sis/MCTD	1	3
Sjs/RA	0	5
Sjs/APS	0	1
SLE/APS	0	5
SLE/RA	1	0
SLE/MCTD	0	1
Sjs/SLE/APS	0	1
Sjs/SLE/RA	0	1
Others	0	7

Bold values indicate statistically significant

CHB congenital heart block, *NLE* neonatal lupus syndrome, *SLE* systemic lupus erythematosus, *Sjs* Sjögren syndrome, *RA* rheumatoid arthritis, *APS* Anti-phospholipid syndrome, *MCTD* mixed collagen tissue disease

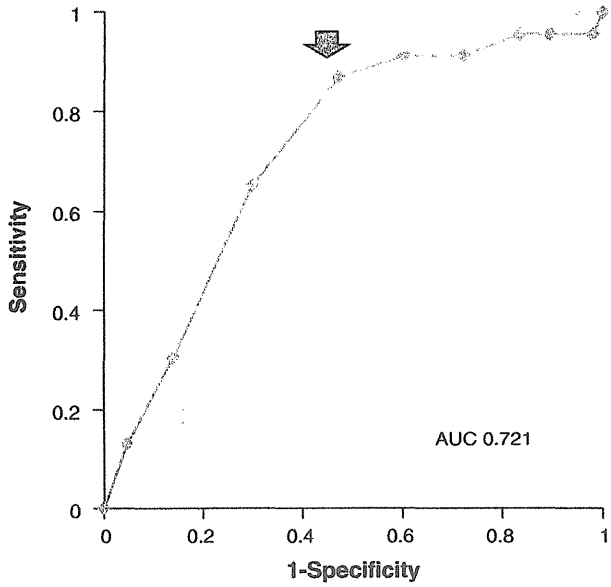

Fig. 1 Receiver-operating characteristics curve for the anti-SS-A antibodies titer in the prediction of fetal CHB *AUC* area under the curve, *arrow* denotes 32 times in DID

Multivariate analysis (Table 3)

We then performed a multivariate analysis using the seven variables significant to fetal CHB. As shown in Table 3, right panel, the odds ratio (95 % confidence interval) for maternal age, and an anti-SS-A antibodies titer of 1:32 or higher were 0.78 (0.62–0.98), $P < 0.05$; 27.77 (1.87–413.44), $P < 0.05$, respectively.

Efficacy of steroid therapy in patients with an anti-SS-A antibodies titer of 1:32 or higher (Tables 3, 4)

Use of steroids was not a predictor of CHB in the multivariate analysis. The association of steroid use with CHB was therefore assessed in the subset of patients with an anti-SS-A antibodies titer of 1:32 or higher (Table 3).

Among 107 patients with an anti-SS-A antibodies titer of 1:32 or higher, 65 (60.7 %) were treated with steroids taken orally during pregnancy; of these, four patients developed CHB (6.2 %). This percentage was significantly lower ($P < 0.01$) than that of the patients not treated with steroids. Among the patients treated with steroids, no patients (0 of 27) treated with prednisolone (dose: median 7.2 range 2.5–12.5 mg) and four of 38 patients treated with betamethasone (initiated at a dose of 2 mg/day at the gestational age of 12–20 weeks, with tapering after 2 weeks) developed CHB.

Fourteen of 41 patients had received prednisolone prior to receiving betamethasone, with the remaining 27 receiving betamethasone only.

Discussion

In this study we investigated risk factors for the development of CHB in anti-SS-A antibodies positive pregnant women. Our main finding was to establish anti-SS-A antibodies titer of 1:32 as the cut-off value based on analysis of a ROC curve. A multivariate analysis showed that an anti-SS-A antibodies titer of 1:32 or higher by DID was an independent risk factor for fetal CHB.

Franco et al. first described the relationship between maternal anti-SS-A antibodies and components of NLE, particularly CHB. Subsequently, several studies have investigated various antibodies including 52 kD SS-A/Ro or 60 kD SS-A/Ro, which do play significant roles. Little, however, is known regarding the relationship between the development of CHB and the anti-SS-A antibodies titer, and whether this relationship is causal.

The method used to titer the antibodies must be considered when assessing results. ELISA is commonly used given that it is simple to perform and automatable. False positive results, however, are common. The present study utilized DID. This method is the standard method for detecting anti-U1RNP, anti-Sm, anti-SS-A, anti-SS-B, anti-Scl-70, anti-Jo-1 antibodies, and is more reliable than the ELISA method [13].

Recently, in a study of 186 fetuses, Jaeggi et al. [8] identified fetal exposure to anti-SS-A levels ≧50 U/ml as significantly increasing the risk of CHB (5 vs. 0 % for <50 U/ml, odds ratio 7.8; range 0.4–159). This study employed enzyme-linked immunosorbent assay (ELISA) measurements. The ELISA assays utilized in the present and prior study were manufactured by different companies, which may explain the discrepancy in the results. Another recent study describes a standardized method for measuring both of the 52 and 60 kD SS-A antibodies, which is more sensitive and accurate than the conventional ELISA kits and the DID method [14, 15]. Thus, it is possible that further investigation using this new assay may confirm the predictive level of anti-SS-A antibodies by ELISA for CHB.

Another finding in this study was that antepartum steroid treatment with either prednisolone or betamethasone, may reduce the risk of fetal CHB in women with an anti-SS-A antibodies titer of 1:32 or higher. Use of either steroid significantly suppressed CHB in comparison to no treatment irrespective of which steroid was selected. Since orally administered prednisolone is inactivated by placental 11 beta HSD type 2 before reaching fetal heart [16], the effect of prednisolone is to diminish a generalized inflammatory insult and to eliminate the candidate maternal autoantibodies. Therefore, it is likely that the mechanism by which steroids affect CHB is maternal rather than fetal [17].

Table 3 Multivariate analysis for fetal CHB

	Odds ratio	95 % confidence interval	*P* value
Maternal age	0.78	0.62–0.98	**<0.05**
Gestational week at delivery	0.72	0.50–1.04	0.08
Apgar score (5 min)	0.83	0.48–1.42	0.49
Anti-SSA antibody titer 32 times or more	27.77	1.87–413.44	**<0.05**
Signs and symptoms of maternal autoimmune disease	0.86	0.13–5.62	0.88
Diagnosis of maternal autoimmune disease	0.27	0.05–1.48	0.13
Medications taken before and during pregnancy	0.2	0.04–1.06	0.06

Bold values indicate statistically significant

Multivariate analysis of seven predictors of fetal CHB was performed using EXCEL Tokei 2010—in Japanese (Shakai Joho Service, Tokyo, Japan). *P* values <0.05 were considered significant

DID double immunodiffusion

Table 4 Efficacy of steroid therapy in patients with an anti-SS-A titer 1:32 or higher

Steroid treatment	CHB		*P* value	
	Positive	Negative		
No steroids	12	30	<0.01	
Steroids, overall	4	62		
Prednisolone	0	25	<0.01*	0.082**
Bethamethazone	4	37	<0.05*	

* Comparison with patient without steroids

** Comparison between patients with prednisolone and betametazone

Autoimmune associated CHB occurs by a two-stage process. In the first step, maternal autoantibodies bind fetal cardiomyocytes, dysregulate calcium homeostasis, and induce apoptosis in affected cells. This step may clinically correspond to a first-degree heart block, and be reversible. As inflammation progresses, as may be the case in genetically susceptible fetuses, progressive tissue damage will lead to fibrosis, calcification of the AV-node and subsequent CHB [18]. It is plausible that the prevention of CHB is likely due, in part, to anti-inflammatory effects in the fetus. It is likely that suppression of the maternal autoimmune component also plays a role.

Patients with SLE or Sjögren Syndrome exhibit asymptomatic inflammation and fluctuations in the levels of numerous inflammatory cytokines [19]. NF-kappa B promotes a chronic inflammatory response through regulating the expression of genes involved in immuno-inflammatory responses, cell cycle progression, inhibition of apoptosis, and cell adhesion [20]. These inflammatory processes may represent the target of prophylactic prednisolone in SLE mothers. Although the mothers with CHB fetuses were similar to the mothers without affected fetuses in terms of significant obstetrical history including a prior history of CHB in a fetus, it was not possible in the present study design to control for factors related to disease severity. Therefore, it is not possible from the present study to ascertain whether maternal disease modification by steroid treatment was directly related to a decreased risk of fetal heart block.

In our retrospective study, betamethasone was given to 41 patients with individual informed-choice base. Each patient was counseled with the understanding that not only has its efficacy in the prevention of fetal CHB not been established, but there are also possible adverse effects for the mother, including mood disorder, insomnia, and increased appetite, adverse obstetric events such as spontaneous abortion, stillbirth, neonatal adrenal insufficiency and long-term brain development. The protocol was not unified, but basically followed antecedent case reports [21, 22]. In brief, betamethasone was given from around 12 to 26 weeks of gestation, initiated at a dose of 2 mg/day, tapered every 2–4 weeks.

Transplacental steroid treatment carries potential risks. The major concerns with chronic steroid use are negative effects on neurological development, growth retardation, and oligohydramnios as well as hypertension, diabetes, infection, and osteonecrosis and osteoporosis in the mother. Fluorinated steroids have, in both human neonates and animal models, been shown to affect intrauterine growth and the central nervous system development with either single or repeated doses [18, 23]. It is unclear, however, whether the results of these studies are directly applicable to the fetus with CHB. Hutter et al. suggest that the risks of high-dose transplacental steroid treatment are in part avoidable by lowering the dexamethasone dosage [24]. As prednisolone was shown to have an equivalent effect in our study population, it is the preferable formulation as its adverse effects are generally considered acceptable even in early pregnancy [25]. A large, prospective study is necessary to ascertain the effectiveness and safety of prednisolone to prevent fetal CHB in patients with anti-SS-A antibodies titer of 1:32 or higher.

In this study, we found that an anti-SS-A antibodies titer of 1:32 or higher by DID was an independent risk factor for fetal CHB. In these patients, either prednisolone or betamethasone, during pregnancy might reduce fetal CHB. These findings may provide a new clinical strategy to prevent fetal CHB in combination with PR measurements and conventional approaches.

Acknowledgments We thank Ms. Takagai and Mr. Kamakura for their technical assistance. This work was supported in part by a grant-in-aid from the Japan Ministry of Health and Labour (H22-Jisedai-Ippan-007), the Japan Ministry of Education (23591596) and "FUKUOKA" OBGYN Researcher's Charity Foundation Fund.

Conflict of interest None.

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Fetal Heart Rate Predictors of Long QT Syndrome

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Background—Fetal long QT syndrome (LQTS) is associated with complex arrhythmias including torsades de pointes and 2° atrioventricular block. Sinus bradycardia has also been associated with fetal LQTS, but little is known of this rhythm manifestation. Our purpose was to characterize the fetal heart rate (FHR)/gestational age (GA) profile of fetal LQTS.

Methods and Results—We ascertained fetal LQTS subjects by family history (Group 1) or fetal arrhythmia referral (Group 2). We compared FHR in LQTS subjects versus normal fetuses. To identify FHR predictors of LQTS, we calculated a bradycardia index as % of LQTS FHR recordings either ≤110 beats per minute (obstetric standard) or ≤3rd percentile for GA. Among 42 LQTS subjects, 26 were in Group 1 and 16 in Group 2. There were 536 normal fetuses. The bradycardia index was only 15% for FHR ≤110 beats per minute, but 66% for FHR ≤3rd percentile for GA. Ten fetuses with complex arrhythmias also had severe and sustained sinus bradycardia throughout gestation. Identifying a fetal proband in Group 2 resulted in LQTS diagnosis in 9 unsuspected members of 6 families.

Conclusions—FHR varies by GA in both normal and LQTS fetuses. Postnatal evaluation of neonates with FHR ≤3rd percentile for GA may improve ascertainment of LQTS in fetuses, neonates, and undiagnosed family members. (***Circulation.*** **2012;126: 2688-2695.)**

Key Words: arrhythmias, cardiac ■ fetus ■ long-QT syndrome

Long QT syndrome (LQTS) is reported to have an incidence of 1 in 2500 individuals. Although QT interval prolongation may be an incidental finding, LQTS typically presents in adolescence or young adult life with syncope, sudden death, or cardiac arrest.[1,2] Less frequently, LQTS presents in the perinatal (fetal/neonatal) period; in this setting morbidity and mortality are high, and torsades de pointes (TdP) and 2° atrioventricular (AV) block are signature rhythms.[3–6] Sinus bradycardia is also a manifestation of fetal LQTS and is reported to be more common than TdP and 2° AV block. For example, as many as 44% to 66% of fetuses diagnosed with LQTS presented with sinus bradycardia at 26 to 40 weeks of gestation.[3,6–8] In most reports, a fetal heart rate (FHR) ≤110 beats per minute (bpm) at any gestational age (GA) raised suspicion of LQTS. Indeed, FHR of ≤110 bpm at any GA is the obstetric definition of sinus bradycardia.[9] However, little is known of the sensitivity of this finding and how it relates to the subsequent diagnosis of LQTS.

Clinical Perspective on p 2695

It is well known that FHR in the normal fetus decreases during gestation from about 175 bpm at 10 weeks to 138 bpm at 40 weeks. This phenomenon is believed to be attributable to the increasing dominance of the parasympathetic nervous system on heart rate control as gestation progresses.[10,11] Despite the association between fetal bradycardia and LQTS, the FHR/GA profile, or the range of FHRs of subjects with LQTS, has not been defined. We wondered whether there might be a pathological FHR in the setting of 1:1 AV conduction that was below normal for GA but >110 bpm that might be a sensitive marker for fetal LQTS. We hypothesized that the FHR/GA profile of LQTS individuals would be different than that of normal individuals and there might be GA-specific or genotype-specific FHR predictors of LQTS.

The purposes of this study were first to define the FHR/GA and rhythm profile of individuals with LQTS mutations and compare this profile with that of a normal control group. Second, we hoped to develop FHR criteria that would improve the recognition of LQTS in the perinatal period.

Methods

This was a study of fetal cardiac rhythm in pediatric subjects with a clinical and genetic diagnosis of LQTS. Participants were

Received May 25, 2012; accepted October 12, 2012.

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The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.112.114132/-/DC1.

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Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.112.114132**

recruited at 3 medical centers (Advocate Hope and Lutheran General Children's Hospitals, Chicago IL; University of Tsukuba, Tsukuba, Ibaraki, Japan; and University of Utah and Primary Children's Medical Center, Salt Lake City, UT). Fetal heart rate in LQTS subjects across GA were compared with FHR from a normal control group across similar GA. Approval from the institutional review boards of each participating center was obtained.

Study Groups

Recruitment of LQTS Subjects

To avoid possible ascertainment bias, we divided LQTS subjects into 2 groups. Subjects in Group 1 had a family history of genetically confirmed LQTS and were under increased surveillance because of a risk of LQTS recurrence. Group 2 consisted of fetuses referred for evaluation of cardiac arrhythmia; in some cases Group 1 subjects were siblings of individuals in Group 2.

Recruitment of Normal Subjects

Normal subjects were recruited from Advocate Christ Medical Center and Hope Children's Hospital. Inclusion criteria were a normal obstetric ultrasound or a normal fetal echocardiogram. Indications for obstetric ultrasound of the normal subjects at 7 to 15 weeks were viability and nuchal translucency screening; indications for echocardiography of the normal subjects at 16 to 40 weeks were maternal diabetes mellitus, advanced maternal age, medication exposure or suspicion of fetal disease (eg, family history of a congenital heart defect). Exclusion criteria for normal subjects at 7 to 40 weeks GA included abnormal nuchal translucency measurement, fetal cardiac arrhythmia or history of arrhythmia, congenital heart defect, or clinically significant noncardiac malformation (eg, spina bifida), or known chromosome abnormality. The FHR of 12 infants evaluated for a maternal or paternal history of LQTS but with negative genetic testing were included in the normal subjects. No subject in the normal Group was related to any subject in LQTS Groups 1 or 2.

Fetal Heart Rate Measurements

LQTS Subjects

FHR of the LQTS subjects were obtained from obstetric records throughout the mother's pregnancy. They were derived from M-mode measurements of ventricular or atrial contractions from 5 consecutive cardiac cycles when the fetus was still, or by Doppler auscultation of the FHR routinely performed at monthly or twice monthly visits to the obstetric care provider. For fetuses with AV block, FHR was determined either during intermittent sinus rhythm or by measuring the atrial rate. Rhythms of LQTS subjects were classified as either sinus or complex, specifically TdP or 2° AV block.

Normal Subjects

Method for determination of FHR in normal fetuses was GA-dependent. FHR at 7 to 15 weeks of gestation were measured from atrial or ventricular M-mode waveforms of 5 consecutive cardiac cycles obtained during routine obstetric ultrasounds. FHR at 16 to 40 weeks of gestation were measured from the aortic or pulmonary valve Doppler waveforms of 5 consecutive cardiac cycles obtained during fetal quiescence. Data were obtained from 10 fetuses for each week of gestation from 7 to 40 weeks.

LQTS Diagnosis

The diagnosis of LQTS was based on findings of a positive genetic test for LQTS. All genetic testing was performed in commercial genetic testing laboratories. Samples were tested for either 12 (GeneDx, Gaithersburg, MD) or 13 (Familion, Transgenomic Inc., New Haven, CT) LQTS gene subtypes. Only genetic variants reported to be deleterious were considered to be mutations; variants reported to be of uncertain significance were not considered pathological. Mutations were classified as LQTS gene type, compound (>1 deleterious mutation), uncharacterized (no mutation in a known LQT gene), or untested. The presence of a signature LQTS rhythm, TdP or 2° AV block, in the fetal or neonatal period was considered confirmatory of the LQTS diagnosis, even if genetic testing revealed no mutation in a known LQTS gene.

The QT interval on a postnatal 12 lead ECG was corrected (QTc) by both Bazett and Frederica formulas and reported for LQTS subjects. Subjects in Group 1 had an ECG at the time of their initial evaluation during infancy or childhood, whereas those in Group 2 had an ECG during the first 24 hours of life.

Fetal Heart Rate Analysis

We determined the 3rd, 50th, and 97th percentiles for FHR/GA of the normal subjects by logarithmic regression analysis. For the purposes of this study, we defined bradycardia in 2 different ways: either independent of GA (FHR $\leq$110 bpm, the obstetric definition of bradycardia) or dependent on GA (FHR $\leq 3^{rd}$ percentile for GA).

Statistical Analysis

The FHR (mean$\pm$SE) was calculated for normal subjects and LQTS subjects. To maximize statistical power, we grouped the FHR data into 3 categorical GA groups, (1) <21 weeks, (2) 21 to 30 weeks, and (3) 31 to 40 weeks, and compared normal and LQTS FHR in the 3 GA groups. A mixed effect model was performed taking the dependency of patients within the same family into account. To eliminate maternal β-adrenergic blockade therapy as a confounding variable for observed FHR differences between normal and LQTS subjects, we compared FHR of treated and untreated mothers with LQTS by Mann–Whitney nonparametric testing. A 2-tailed P level of <0.05 was considered statistically significant. All analyses were done using SAS 9.2 (SAS Inc., Cary, NC).

Bradycardia Index of LQTS Subjects

Once we derived the 3rd, 50th, and 97th percentiles for the normal subjects at each GA, we calculated a bradycardia index for each LQTS fetus using both definitions of bradycardia. In other words, the bradycardia index was the ratio of FHR measures that were either $\leq$110 bpm or $\leq 3^{rd}$ percentile for GA compared with the total number of FHR measures for that fetus. Because the number of subjects in each genotype group was small, we did not seek to define a genotype-specific effect on FHR or bradycardia index within categorical age groups or between Groups 1 and 2.

Results

LQTS Subjects

The descriptions of the LQTS cohorts in Group 1 (referred with a family history of LQTS; n=26) and Group 2 (referred for arrhythmia evaluation; n=16) are summarized in Table 1. Among the 42 subjects, a diagnosis of LQTS was made during fetal or neonatal life in 32 subjects; in 10 subjects in Group 1, the diagnosis was made later during infancy or childhood. The QTc intervals corrected by Bazett formula ranged from 450 to 700 (mean 582) ms and corrected by Frederica formula ranged from 394 to 660 (mean 471) ms. Bazett correction resulted in a prolonged QTc ($\geq$ 450 ms) in 95% of genetically proven LQTS subjects, whereas use of Friderica correction identifies only 59% of genetically proven LQTS subjects as having a prolonged QTc. The corrected QT intervals by both formulae are shown in the Table I in the online-only Data Supplement. Several members of Group 1 were in previously reported families.[12–14] Siblings of 4 families were included in Group 1: subjects #10, #11, and #12;

Table 1. Study Cohort

ID	GA Arrhythmia Detected (wks)	Age Enrolled	GA Delivery (wks)	LQTS Mutation
Group 1: Referral for Family History of LQTS				
*1(12)	...	F/N	38	*KCNQ1* A341V (13)
2	...	F/N	38	Not tested
3	...	F/N	38	Not tested
4	...	F/N	39	*KCNQ1* R594Q (14)
5	...	3 y	41	*KCNQ1* R259C (15)
6	...	2 y	38	*KCNQ1* G314D (16)
*7	...	5 y	39	*KCNQ1* IVS2+5G>A (splice)(17)
*8	...	2 y	37	*KCNQ1* IVS2+5G>A (splice)(17)
9	...	F/N	39	*KCNQ1* A226V (18)
*10(19)	...	F/N	41	*KCNQ1* W305X (19)
*11	...	F/N	37	*KCNQ1* W305X (19)
*12	...	F/N	37	*KCNQ1* W305X (19)
*13	...	F/N	35	*KCNQ1* R190W (20)
*14	...	F/N	31	*KCNQ1* R591H+*KCNE1* S28L (21)
*15	...	3 y	30	*KCNQ1* R591H (21)
*16	...	F/N	40	*KCNH2* A715A (splice) (17)
†17(22)	...	6 y	40	*KCNQ1* G168R (23)
†18	...	F/N	39	*KCNQ1* G168R (23)
19(14)	...	8 y	40	*KCNE1* D76N (14)
20	...	6 y	41	*KCNE1* D76N (14)
21(13)	...	F/N	40	*KCNQ1* V254M (13)
*22	...	F/N	37	*KCNQ1* V254M (13)
23(24)	...	19 mo	39	*KCNQ1* R518X (25)
24(26)	...	5 y	40	*KCNH2* T1945+6C (26)
25(13)	...	F/N	37	*KCNQ1* A341E (13)
26	...	F/N	39	*SCN5A* T1304M (27)
Group 2: Referral for Fetal Arrhythmia				
27(28)	28	F/N	33	*KCNH2* G628S (29)
28(30)	21	F/N	35	*SCN5A* R1623Q (31)
29	35	F/N	38	Uncharacterized
30	27	F/N	38	Uncharacterized
31	25	F/N	37	*KCNQ1* V110fs+132X‡
32	23	F/N	37	*KCNQ1* R259C (15)
33	22	F/N	38	*KCNQ1* G314D (16)
34	29	F/N	39	*KCNH2* W1001X (32)
35	32	F/N	39	*KCNQ1* R539W (33)
36	31	F/N	35	*SCN5A* R1623Q (29)
37	32	F/N	37	*KCNQ1* A226V (18)
38	30	F/N	40	Not tested
39	32	F/N	38	Uncharacterized
40(34)	19	F/N	IUFD	*SCN5A* L409P (34)‡
41	28	F/N	31	*SCN5A* R1623Q (31)
42	28	F/N	31	*SCN5A* R1623Q (31)

Numbers in parenthesis after subject number are references in which subject was previously described; numbers in parenthesis after mutations are references for first description of mutation. LQTS indicates long QT syndrome; No., number; GA, gestational age; FHR, fetal heart rate; wks, weeks; F/N, fetus/neonate; and IUFD, intrauterine fetal demise.

*Mother with LQTS was on beta blocker treatment during entire pregnancy.

†Mother on β-blocker therapy only during 3rd trimester.

‡Novel mutation.

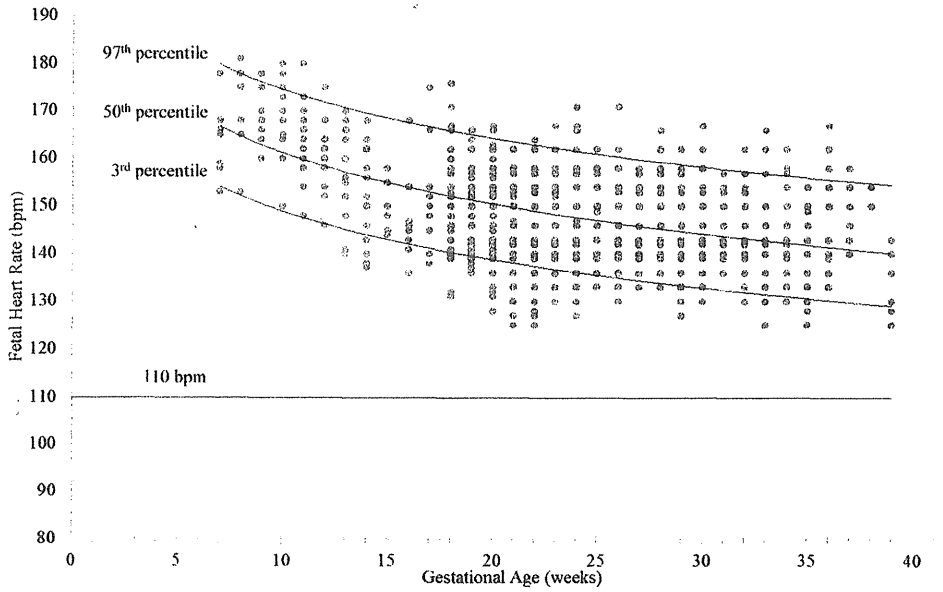

Figure 1. Individual FHR measurements (n=3264 data points) by gestational age of 547 normal fetuses. Curves representing the 3rd, 50th, and 97th percentiles of FHR are shown, as is a horizontal line at 110 bpm, which is the standard obstetric definition of bradycardia. FHR decreases with advancing gestational age. Some normal FHR measurements are <3rd percentile but none are <110 bpm. FHR indicates fetal heart rate; bpm, beats per minute.

subjects #17 and #18, subjects #19 and #20, and subjects #21 and #22. Subjects #41 and #42 in Group 2 were twins. At the time of initial assessment, no Group 2 subject was known to have affected family members; however, subsequent diagnosis in the fetal proband led to a genetic diagnosis of LQTS in undiagnosed members of 6/16 (38%) families. These family members (subjects #5, #6, and #7) were included in Group 1 after diagnosis of LQTS in subjects #32, #33, and #37.

The mean GA at delivery was slightly less for Group 2 (36.4±2.8 weeks) compared with Group 1 (38.0±2.7 weeks) subjects, but this difference was not significant (P=0.08). The mean GA of referral for subjects in Group 2 was 27.6±4.5 weeks. Five subjects in Group 2 were delivered prematurely (≤ 35 weeks of gestation) because of uncontrolled arrhythmia or fetal distress; 1 fetus died in utero from uncontrolled arrhythmia and severe hydrops (Subject # 40).

Among mothers with LQTS, 13 were treated with β-adrenergic blocking agents during pregnancy: 11 throughout pregnancy and 2 during 3rd trimester only. The FHR was not different in fetuses whose mothers were treated (130.1±8.2 bpm) or untreated (127.5±13.6 bpm; P=0.6).

LQTS Mutations in Group 1 Versus Group 2

Mutation in a known LQT gene was found in most subjects (92%) who underwent genetic testing (95%): 23 with LQT1, 4 with LQT2, 6 with LQT3, 2 with LQT5, and 1 with a compound mutation. Three subjects were not tested and 3 subjects had uncharacterized mutations. Among those who had genetic testing, there were differences in genetic results in Group 1 versus Group 2 subjects (Table 1). For example, 83% of Group 1 subjects had a *KCNQ1* or *KCNE1* mutation, 4% had an *SCN5A* mutation, and no subject had an uncharacterized mutation. In contrast, 33% of Group 2 subjects had a *KCNQ1* mutation, 33% had an *SCN5A* mutation, and nearly 20% had uncharacterized mutations (n=3).

Fetal Heart Rates

Normal FHR data from 7 to 40 weeks were obtained from 3264 FHR measurements in 547 normal subjects. The 3rd, 50th, and 97th percentiles for GA are shown in Figure 1. We obtained 318 FHR measures from 42 LQTS fetuses; the mean of FHR measures was ≈8 per fetus and the range was 1 to 12 per fetus. The mean FHR at each of the 3 GA groups (<21 weeks, 21–30 weeks, and 31–40 weeks) was significantly different between normal and LQTS subjects (P<0.001; Table 2). The FHR at the 3rd percentile of normal from the 3 GA groups were all greater than the standard obstetric definition of fetal bradycardia (ie, FHR ≤110 bpm).

We evaluated the individual FHR measures across GA of fetuses based on indication for referral. Figure 2A shows the FHR/GA profile of subjects referred for a family history; Figure 2B shows the FHR/GA profile of subjects referred for evaluation of fetal rhythm. The FHR of the LQTS fetuses decreased with GA as seen in the normal

Table 2. Mean FHR of Normal and LQTS Subjects by GA Group

GA Group	Normal Subjects FHR Mean±SE (bpm)	LQTS Subjects FHR Mean±SE (bpm)	P
<21 wk	152.0±0.4	139.9±1.2	<0.001
21 to 30 wk	146.7±0.2	127.4±1.2	<0.001
31 to 40 wk	143.3±0.4	123.0±1.4	<0.001

LQTS indicates long QT syndrome; GA, gestational age; FHR, fetal heart rate; SE, standard error; and bpm, beats per minute.

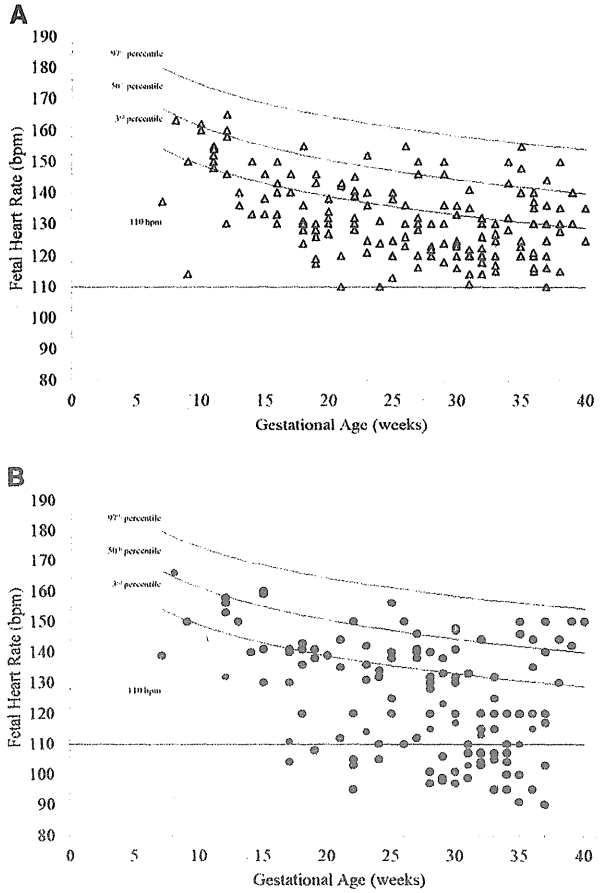

Figure 2. Individual measurements throughout gestation of LQTS fetuses, based on indication for referral. A, FHR of individuals referred because of a family history of LQTS (Group 1). B, FHR of individuals referred for evaluation of fetal rhythm (Group 2). For reference, the line marking a FHR of 110 bpm across gestation is shown in both panels. FHR indicates fetal heart rate; LQTS, long QT syndrome; and bpm, beats per minute.

fetal cohort, but GA-dependent changes in mean FHR differed between Groups 1 and 2. Early, at <21 weeks of gestation, mean FHRs were not different (141.54±2.02 versus 136.95±2.51; $P=0.15$). However, FHR in Group 2 was lower at both 21 to 30 weeks (130.28±2.66 versus 124.54±2.65; $P=0.04$) and at 31 to 40 weeks (127.72±2.81 versus 117.94±2.83; $P<0.01$). Only subjects in Group 2 had FHR ≤110 bpm.

The Bradycardia Index

Among LQTS subjects, only 15% of FHR readings were ≤110 bpm whereas 66% of the FHR readings were ≤3rd percentile for GA. Thus, 85% of the total FHR readings were higher than the standard obstetric definition of bradycardia (FHR ≤110 bpm), and only 33% of the LQTS FHR readings were >3rd percentile for GA. Using FHR ≤3rd percentile for GA, 38% (16/42) of LQTS fetuses had a bradycardia index of 100% and 67% (28/42) had a bradycardia index between 75% to 100%. Table 3 shows there were significant differences between Groups 1 and 2 in the bradycardia indices for FHR ≤110 bpm and ≤3rd

Table 3. Bradycardia Index of Group 1 and Group 2 Based on GA Group

	FHR≤110 bpm		FHR<3rd Percentile for GA	
GA (Weeks)	Group 1	Group 2	Group 1	Group 2
Overall	1%	31%	50%	68%
<21	0%	6%	48%	45%
21 to 30	2%	26%	61%	68%
31 to 40	2%	49%	42%	81%

LQTS indicates long QT syndrome; GA, gestational age; FHR, fetal heart rate; and bpm, beats per minutes.

percentile for GA. Within Group 2, a bradycardia index of 100% was seen in 2/3 subjects with complex rhythms (Table 4). The findings of more pronounced bradycardia in Group 2 subjects with complex rhythms may be another manifestation of a more severe phenotype in such fetal LQTS subjects.

Although the sample sizes of certain LQTS mutations were small, among genotypes, the bradycardia index for FHR ≤3rd percentile for GA was highest (100%) for uncharacterized mutations and lowest (0%) for LQT5 mutations. Overall, the severity of the bradycardia index was not predicted by the presence of mutations in known LQTS genes.

Fetal Heart Rhythms and FHR

In most of the 42 fetuses, sinus rhythm was observed throughout pregnancy, but in 10 fetuses, 8 of whom were in Group 2, complex arrhythmias characterized by 2° AV block or TdP were observed (Table 4). The mean FHRs of these 10 subjects were lower across GA than those subjects who manifested only fetal bradycardia (120.74±3.56 versus 130.79±2.37; $P<0.01$), and the bradycardia indices for FHR ≤3rd percentile were higher (80% versus 60%).

Discussion

There are several novel and clinically relevant findings in this study of fetal LQTS. First, as in normal fetuses, the FHR of LQTS subjects trend downward but are generally lower than FHR of normal fetuses as gestation progresses. Second, there are GA-dependent FHR predictors of LQTS; for example, when compared with a GA independent FHR predictor (FHR ≤110 bpm), a FHR ≤3rd percentile for GA improves ascertainment of LQTS subjects from 15% to 85%. Third, there are shades of bradycardia within the LQTS population: compared with subjects who remained in sinus rhythm during pregnancy, subjects with the lowest FHRs were more likely to have had a complex arrhythmia including TdP or 2° AV block, and were more likely to have de novo or uncharacterized mutations. Together, findings from this study should improve ascertainment of fetal LQTS at all GA.

From the first ultrasound visualization of the fetal heart signifying a viable pregnancy to the reactive accelerations signifying fetal well-being during labor and delivery, FHR is the most frequently and thoroughly evaluated parameter

from the beginning to the end of pregnancy. Yet, neither the range of normal nor the definitions of abnormal FHR are GA-specific, emphasizing the shortcomings of a single definition of fetal bradycardia (eg, FHR ≤110 bpm). Sinus bradycardia occurs in LQTS, but the molecular basis for this common occurrence is incompletely understood. Furthermore, the GA at which the sinus beat becomes bradycardic, and indeed the sensitivity and specificity of a GA-independent definition of bradycardia, are poorly understood.

Previous publications have described a range of sinus FHRs ranging from <100 to 130 bpm in LQTS fetuses.[7,3,4] As in our series, many of these LQTS fetuses with FHRs in the normal range (> 110 bpm) had a family history of LQTS. The higher FHR in those with a family history may be ascertainment bias as these subjects, screened preemptively, may be less severely affected. After birth, the majority of subjects, even those with FHR ≤110 bpm, had heart rates in the normal range.[7] Similarly, in a large study evaluating LQTS and SIDS, bradycardia in the neonate was not considered a risk factor for LQTS.[35] Thus, use of a stringent fetal bradycardia definition (ie, FHR ≤110 bpm) may result in failure to recognize fetal LQTS, and continuation of mild bradycardia (ie, heart rate >110 bpm) after birth may fail to raise the suspicion of LQTS in the neonate. This may explain why the older siblings of some fetal probands in this study with mild bradycardia were not suspected as fetuses or neonates to have LQTS.

In the absence of a known family history, the ascertainment of fetal LQTS is based on the correct and timely diagnosis of the signature LQTS rhythms. Although TdP and 2° AV block are usually easily recognized and have high specificity, they occur infrequently in the fetus with LQTS. For example, only 24% of our study cohort had these complex arrhythmias, and none of the 25 subjects in this report with LQT1 mutations had TdP or 2° AV block. Thus, it is important to identify other markers of LQTS; findings in our study suggest that FHR may be useful for this purpose. Our results show that a one size fits all FHR indicator of bradycardia will not be adequate. For example, the bradycardia index of LQT 1 subjects for FHR ≤110 bpm was only 2%, but definition of bradycardia as FHR ≤3rd percentile yielded a bradycardia index of 68%. Using a GA-independent definition of bradycardia would not have led to suspicion of LQTS in many such subjects.

Although our study group is relatively small, we found associations between FHR, rhythm phenotype, and genotype, which could be helpful in the diagnosis of LQTS. For example, individuals with *KCNQ1* mutations tended to have a mild phenotype in utero with sinus rhythm and mild bradycardia. On the other hand, genetically elusive subjects, with no known mutations and a negative family history of LQTS, had profound fetal bradycardia and complex rhythms.

Based on the results of this study, we believe that FHR ≤3rd percentile for GA is a superior definition of fetal bradycardia compared with the widely used obstetric definition. Our study suggests that the fetus with repeated FHR measurements ≤3rd percentile for GA without any other rhythm abnormality should be suspected of having LQTS. This suspicion should lead to detailed family history for LQTS. Regardless of family history, a postnatal 12-lead ECG should be examined for findings of LQTS. If the family history is positive, or the fetal proband manifests complex LQTS rhythms, ECG screening of first-degree relatives is recommended. Even if family members are asymptomatic, ECG evidence of LQTS warrants genetic testing. Finally, if postnatal genetic testing of the fetus with suspected LQTS is positive, but clinical or genetic manifestations of LQTS are negative in first-degree relatives, the possibility of parental mosaicism should be considered, especially if future pregnancies are contemplated.[36]

Table 4. Complex Fetal Rhythms in Relation to Bradycardia Index in LQTS Cohort

ID	LQTS Mutation	Fetal Rhythm	% FHR Readings ≤3rd Percentile GA	% FHR≤110 bpm
Group 1: Referral for Family History of LQTS				
2	Untested	2° AVB	100	100%
17	KCNQ1-G168R	2° AVB	100	0%
Group 2: Referral for Fetal Arrhythmia				
27	KCNH2-G628S	TdP and 2° AVB	100	84%
28	SCN5A-R1623Q	TdP and 2° AVB	100	75%
30	Uncharacterized	2° AVB	100	92%
33	KCNQ1-G314D	2° AVB	86	0%
36	SCN5A-R1623Q	TdP and 2° AVB	71	14%
40	SCN5A-L409P	TdP	100	0%
41	SCN5A-R1623Q	TdP and 2° AVB	22	0%
42	SCN5A-R1623Q	TdP and 2° AVB	11	0%

LQTS indicates long QT syndrome; TdP, torsade de pointes; AVB, atrioventricular block; GA, gestational age; and FHR, fetal heart rate.

Acknowledgments

We thank Jeannette Barczak, RN for data collection. We also acknowledge the following maternal-fetal medicine physicians for patient referral: Jude Duval, MD, Farhan Hanif, MD, Steven Ambrose, MD, and Jim Keller, MD.

Sources of Funding

This investigation was supported by the University of Utah Study Design and Biostatistics Center, with funding in part from the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through grant 8UL1TR000105 (formerly UL1RR025764).

Disclosures

None.

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CLINICAL PERSPECTIVE

Long QT syndrome (LQTS) may be as common as 1/2500 individuals, yet fewer than 100 cases have been recognized during fetal life. Fetal torsades de pointes and 2° AV block are easily attributed to LQTS. However, these complex arrhythmias are present in only 25% of fetal LQTS; the majority of LQTS fetuses have asymptomatic bradycardia that may not be recognized as an LQTS marker due to its subtle features. The standard obstetrical definition of bradycardia is fetal heart rate (FHR) ≤ 110 bpm. To improve recognition of perinatal LQTS we evaluated the FHR/gestational age (GA) relationship of fetal LQTS mutations versus a normal control group. We found GA dependent FHR predictors of LQTS; for example, when compared to a FHR of 110 bpm at any GA, a FHR ≤3rd percentile for GA improves ascertainment of LQTS subjects from 15 to 85%. Fetuses with the lowest FHR tended to have de novo and genetically elusive LQTS mutations, and in addition to bradycardia, also manifested complex LQTS rhythms. Identification of LQTS in the fetus with a heart rate of <3rd also led to diagnosis of LQTS in unsuspecting family members. Thus, postnatal evaluation of individuals with a FHR ≤3rdpercentile for GA improves ascertainment of LQTS both before and after birth.

Original Paper: Techniques

Fetal Diagnosis and Therapy

Fetal Diagn Ther 2012;32:109–115
DOI: 10.1159/000331399

Received: January 21, 2011
Accepted after revision: August 2, 2011
Published online: May 22, 2012

Comparison of PR Intervals Determined by Fetal Magnetocardiography and Pulsed Doppler Echocardiography

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Key Words
Fetal magnetocardiography · PR interval · Fetal echocardiography · Pulsed Doppler echocardiography

Abstract
Objective: In clinical practice, measurement of mechanical PR interval (mPR) with pulsed Doppler echocardiography is a standard method used to estimate the atrioventricular conduction time in the fetus. However, fetal echocardiography does not directly reflect the electrical properties of the heart. Technological advances in fetal magnetocardiography (fMCG) have allowed recording of the electrical PR interval (ePR) with high time resolution. The aim of this study was to clarify the differences between ePR and mPR. ***Methods:*** The study subjects were 295 normal human fetuses (gestational age, range 20.4–41.4 weeks) who underwent fMCG, and 135 of them underwent fetal echocardiography 15–90 min before or after fMCG. The ePR was measured using the fMCG, and the mPR was determined by two pulsed Doppler methods, simultaneous recording of the left ventricular inward and outward flow (LV in/out) (n = 135) and superior vena cava and ascending aorta (SVC/aAo) (n = 84). ***Results:*** The ePR showed a significant, but weak, positive correlation with gestational age ($r = 0.162$, $p = 0.0053$). The mPR was significantly longer than the ePR ($p < 0.0001$), with mean differences of 14.6% (95% limits of agreement −10.7, 39.9) for the LV in/out method and 14.7% (95% limits of agreement −8.6, 38.0) for the SVC/aAo method. ***Conclusion:*** Our results point to the risk of overestimation of the atrioventricular conduction time when the mPR is used, and the need for careful interpretation of PR prolongation determined by mPR.

Introduction

Assessment of the atrioventricular conduction time is important in the analysis of fetal arrhythmia. After birth, the PR interval on the surface electrocardiogram is used as a simple parameter of the atrioventricular conduction time. Technological advances in fetal magnetocardiography (fMCG) have enabled acquisition of fetal PQRST waveforms, and standardized values of the fetal electrical RR interval (eRR) and electrical PR interval (ePR) have been proposed [1–5]. However, the fMCG systems were designed for research-based use with limited clinical application. In 2003, the Japanese Ministry for Health, La-

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bor, and Welfare approved the clinical use of the Hitachi model 64-channel magnetometer (Hitachi High-Technologies Corporation, Tokyo, Japan) for fMCG. In 2008, clinical application of fMCG under the health insurance scheme started at Tsukuba University Hospital.

Fetal ultrasound cardiography (fUCG) is the most widely used modality to estimate the fetal atrioventricular conduction time [6, 7]. Measurement of the interval between the onset of blood flow induced by atrial contraction (A wave) and that of ventricular contraction (V wave) by pulsed Doppler allows estimation of the mechanical PR interval (mPR). So far, several pulsed Doppler methods have been used to measure the mPR, including simultaneous sampling of inward and outward flow of the left ventricle (LV in/out) [8, 9], reverse flow in the superior vena cava and forward flow in the ascending aorta (SVC/aAo) [10], and reverse flow in the pulmonary vein and forward flow in the pulmonary artery [11]. Pulsed Doppler-derived mPR seems to be a good surrogate for ePR. However, two reports [12, 13] that focused on the relationship between mPR and ePR measured on the signal-averaged fetal electrocardiogram demonstrated overestimation of the mPR. In the assessment of mPR in the fetus, consideration of the difference between mechanical and electrical properties is important.

The aim of this study was to clarify the difference between ePR determined by fMCG and mPR by two pulsed Doppler methods.

Material and Methods

Subjects

Between April 2008 and September 2010, 295 healthy normal human fetuses underwent fMCG. Of those, 135 fetuses underwent fUCG 15–90 min before or after fMCG. Doppler recording was performed in 135 fetuses with the LV in/out method, and in 84 fetuses the SVC/aAo method was also used. Fetuses with structural heart disease, arrhythmia, or maternal disease (e.g. diabetes mellitus, autoimmune diseases) were excluded from the study.

Instrumentation and Measurements

A 64-channel superconducting quantum interference device (SQUID) system (MC-6400, Hitachi High-Technologies Corporation) housed in a magnetically shielded room was used in this study. This system was designed for use in both adults and fetuses. The SQUID sensors were distributed at 8 × 8 points with an interval of 25 mm, covering an area of 175 × 175 mm. The co-axial 64 sensors detect the tangential component of magnetic fields generated by the fetal heart. Magnetic signals were acquired at a sampling rate of 1,000 Hz for 120–240 s, and the signals were passed through a band-pass filter (0.1–100 Hz) and a power-line noise filter (50 Hz). The fMCG was recorded by positioning the maternal abdominal surface to the sensor as close as possible. Immediately before recording, the fetal heart position and the distance between the maternal abdominal surface and the anterior surface of the fetal ventricle were determined by a portable echocardiographic machine. The fMCG was recorded 2–4 times in one session, with different maternal positions if necessary. The ePR and eRR were measured at the baseline HR beats, and the averaged values of more than 3 consecutive beats were calculated in this study (fig 1a). The onset and offset of the P wave, QRS complex, and T wave were determined manually using MCG analysis software (Hitachi High-Technologies Corporation). Recording and measurement of fMCG was performed by a single observer (Y.K.) without knowledge of the fUCG findings. Two-dimensional and pulsed Doppler fUCG were performed in each case using the SONOS-5500 (Philips, Andover, Mass., USA) for the screening of structural heart disease and measurement of parameters, and the acquired pulsed Doppler waveforms were stored in the optical disk recording system. The mPR and mechanical RR interval (mRR) were determined by the LV in/out methods and the SVC/aAo method was also used if time permitted. LV in/out was recorded by placing the Doppler sample volume at the junction of the anterior leaflet of the mitral valve and left ventricular outflow tract in an apical 5-chamber view, by simultaneous recording of the inflow and outflow of the left ventricles (fig. 1b) [8, 9]. The SVC/aAo was recorded by placing the Doppler sample at the point where the SVC and aAo adjoined each other (fig. 1c) [10, 14, 15]. Recording and measurement of fUCG was performed by another single observer (M.T.-I.) who did not know the fMCG findings. Due to limitations of time in our outpatient clinic, all of the fUCG examinations, including screening for structural heart diseases, acquisition of the Doppler waveforms, and measurement parameters, were performed within 20 min.

Interobserver and Intraobserver Variations

For assessment of interobserver variation, certain parameters were measured again in 30 randomly selected fetuses by another observer (T.I.) for fMCG and (Y.K.) for fUCG. For assessment of intraobserver variation, the first observer remeasured the ePR and mPR in 30 randomly selected fetuses with a time interval of more than 3 months.

Statistical Analysis

Continuous values are expressed as means ± SD. Student's unpaired t test was used to assess differences in gestational age between the fetuses in whom mPR could be determined and those in whom it could not. The correlations between ePR and gestational age, as well as eRR and gestational age, were computed using linear regression analysis. Stepwise multiple regression analysis was used to assess the effect of eRR, estimated fetal body weight, and gestational age on ePR. Direct comparisons between ePR and mPR were made using Student's paired t test. Bland-Altman analysis was used to display the bias and the limits of agreement between ePR and mPR [16]. Stepwise multiple regression analysis was used to assess the effect of gestational age, eRR, and differences between eRR and mRR on the differences between ePR and mPR. Bland-Altman analysis was also used for assessment of interobserver and intraobserver variations. $p < 5\%$ and $F > 2.0$ was considered statistically significant. All statistical analyses were performed using StatView 5.0 software (SAS Institute, Inc., Cary, N.C., USA).

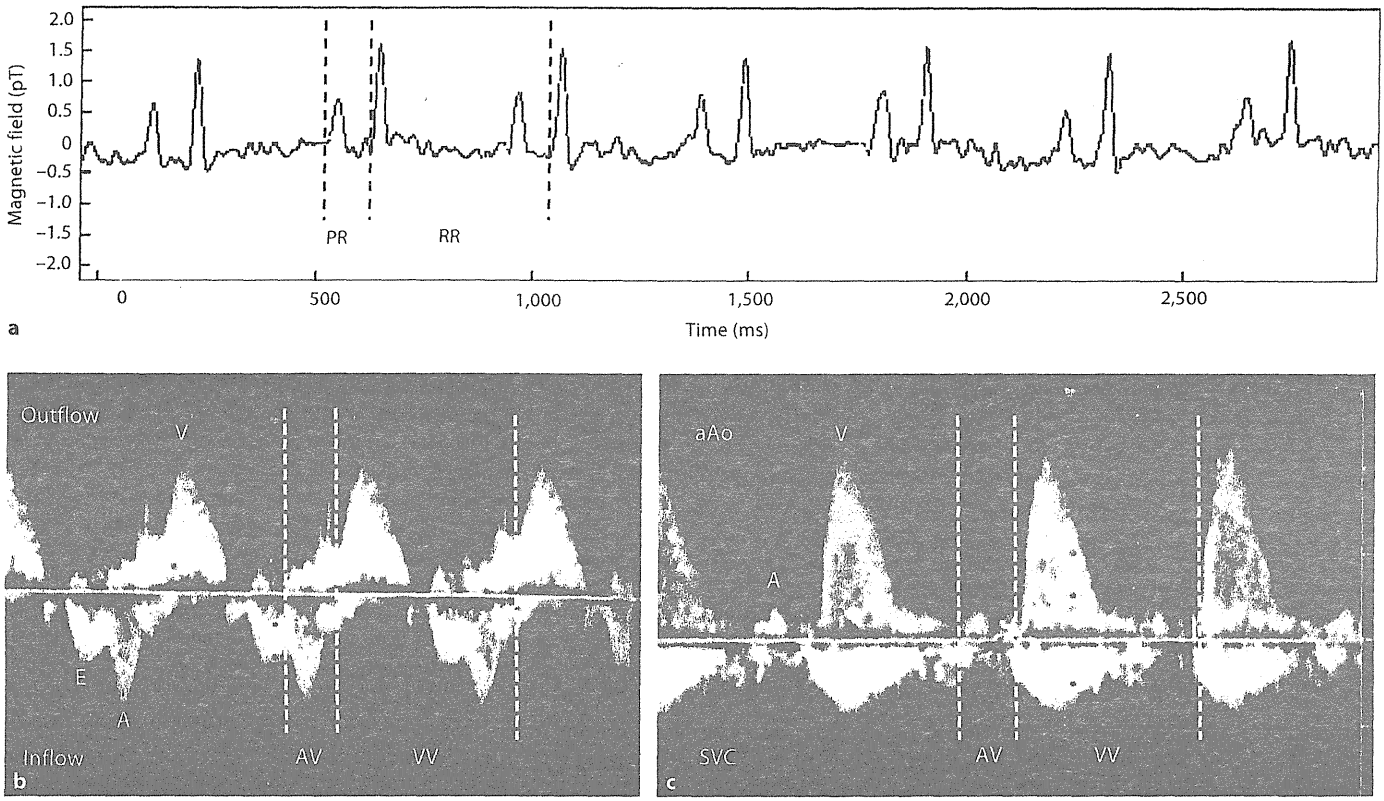

Fig. 1. Measurement of PR and RR intervals. **a** Waveforms of raw data of fMCG. The ePR represents the interval between the onsets of the P wave and the QRS complex, while the RR interval represents the interval between the onsets of two consecutive QRS complexes. **b** Waveforms of simultaneous recording of inward and outward flow of the left ventricle by the pulsed Doppler method. The mPR represents the interval between the onsets of the A wave of inflow and the V wave of outflow. **c** Waveforms of simultaneous recording of bidirectional flow of the SVC and forward flow of the aAo by the pulsed Doppler method. The mPR represents the interval between the onsets of the reversal A wave in SVC and the V wave of aAo.

Results

The success rates of determination of PR intervals were 100% with fMCG (n = 295), 88.9% with the LV in/out method (n = 135), and 94.0% with the SVC/aAo method (n = 84). The mean ePR was 100.2 ± 15.7 ms (range 64–143). The mean mPR was 119.6 ± 12.3 ms (range 85–150) in the LV in/out method and 120.2 ± 12.1 ms (range 93–150) in the SVC/aAo method. The success rate of determination of the PR interval and the distribution of the gestational age of fetuses are summarized in table 1. There were no significant differences in gestational age between the fetuses in whom mPR could be determined and those in whom it could not for both the LV in/out method (p = 0.71) and the SVC/aAo method (p = 0.85). A significant, but weak, positive correlation was noted between ePR and gestational age (r = 0.162, p = 0.0053), and between eRR and gestational age (r = 0.232, p < 0.0001) (fig. 2a, b). Stepwise multiple regression analysis indicated that only gestational age significantly but weakly affected ePR (r = 0.159, F = 6.134).

Table 1. Distribution of the gestational age of fetuses and success rate of determination of PR intervals

Gestational age	ePR (%)	mPR (%)	
		LV in/out	SVC/aAo
≤28 weeks	71/71 (100)	39/41 (95.1)	24/26 (92.3)
29–35 weeks	155/155 (100)	70/80 (86.3)	43/45 (95.6)
36–42 weeks	69/69 (100)	12/14 (85.7)	12/13 (92.3)
Overall	295/295 (100)	120/135 (88.9)	79/84 (94.0)

The results of paired comparisons of ePR with mPR, and eRR with mRR, are summarized in tables 2 and 3.