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Factors influencing fetal cardiac conduction in anti-Ro/SSA-positive pregnancies

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Abstract

Objectives. Congenital heart block (CHB) develops in 1–2% of anti-Ro/SSA-positive pregnancies and has a recurrence rate of 12–20%, which indicates that factors other than maternal autoantibodies are crucial for CHB to occur. Here, we aimed to evaluate the influence of factors previously associated with CHB on the occurrence of milder forms of fetal cardiac conduction disturbances, shown to occur in up to 30% of anti-Ro/SSA-positive pregnancies, and on neonatal outcome in a large cohort of prospectively followed pregnancies.

Methods. The association of maternal age, season of the year and history of atrioventricular block (AVB) with the development of fetal Doppler and neonatal ECG conduction disturbances was evaluated in 212 anti-Ro52/SSA-positive singleton pregnancies.

Results. Maternal age was significantly higher in AVB II–III pregnancies but was not correlated with fetal AV time intervals in fetuses without signs of AVB II–III. AV time intervals of fetuses surveilled during the winter were significantly longer than those of fetuses surveilled during the summer. Fetal AV time intervals in consecutive pregnancies from the same women were significantly correlated. A history of AVB II–III was associated with significantly longer AV time intervals, and AVB I–III was observed at birth in 38% of babies born after a sibling with abnormal fetal AV conduction.

Conclusion. Our study shows that AV time intervals in anti-Ro/SSA antibody-exposed fetuses during the CHB risk period are influenced by the season of the year, and reveals that the recurrence of conduction disturbances in antibody-exposed fetuses is higher than previously reported when milder forms are taken into account.

Key words: congenital heart block, Ro/SSA, autoantibodies

CLINICAL SCIENCE

Rheumatology key messages

- Atrioventricular block II–III is more likely to develop in the fetus of older mothers.
- Atrioventricular conduction time in anti-Ro/SSA autoantibody-exposed fetuses is influenced by the season of the year.
- Taking into account milder forms, recurrence of conduction disturbances in autoantibody-exposed fetuses is higher than previously reported.

Introduction

The association of anti-Ro/SSA autoantibodies, which are commonly found in women with systemic rheumatic diseases such as SS and SLE, with the development of congenital heart block (CHB) is well recognized [1–3]. However, although these antibodies are present in ~90% of pregnancies with CHB diagnosed *in utero* [4], the risk for complete third-degree atrioventricular block (AVB) in anti-Ro/SSA antibody-exposed fetuses is only

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1–2% [5–7]. In addition, several studies have shown that the risk for CHB recurrence in subsequent pregnancies is 12–20% [8–12], despite persistence of maternal autoantibodies, indicating that other maternal, fetal or environmental factors play a substantial role in CHB development.

Most cases of CHB are identified when the fetus presents with bradycardia and examination reveals the presence of a 2:1 second- or third-degree AVB (AVB II–III). However, surveillance programmes following anti-Ro/SSA antibody-positive women with serial Doppler echocardiography during the CHB risk period (weeks 18–24 of pregnancy) have revealed that a substantial proportion of antibody-exposed fetuses have abnormal, significantly prolonged Doppler-derived atrioventricular (AV) time intervals during the CHB risk period [13, 14]. Although the potential association of various factors with AVB II–III has been investigated [8, 11, 15, 16], the impact of these factors on the development of subtler or more transient fetal cardiac conduction disturbances has not been evaluated. The aim of this study was therefore to investigate the influence of factors previously associated with second- or third-degree AVB, such as maternal age, season of the year and AVB history in preceding pregnancies, on *in utero* AV time intervals and fetal outcome in a large cohort of 212 prospectively followed anti-Ro/SSA antibody-positive pregnancies.

Methods

From 2000 to 2015, 212 anti-Ro52/SSA antibody-positive singleton pregnancies in 155 women were followed during the mid-trimester at our tertiary referral centre of fetal cardiology with the aim of early detection of progressive conductive disease followed by immediate therapeutic intervention. Written informed consent was obtained from all participating individuals, and the study was approved by the regional ethics committee, Stockholm North.

Our protocol of weekly fetal echocardiographic examinations during weeks 18–24 of pregnancies at risk of CHB as well as our methods and criteria for diagnosing AVB have been described previously [13, 14, 17]. In the present study, we used time intervals on Doppler flow recordings from left ventricular in- and outflows (MV-Ao) as an estimate of electrical AV conduction. Recordings from the superior vena cava and aorta were also performed, with very similar results, but are not reported. All MV-Ao time intervals were measured by a single investigator on three Doppler waveforms on a digital stored image and averaged. As MV-Ao intervals increase slightly during gestation, all time intervals were converted to *z*-scores by using reference values constructed from 339 normal pregnancies [18]. For each pregnancy, the highest average of two MV-Ao values recorded over two consecutive weeks during the CHB risk period was used for analysis. As data collection was performed over 15 years, we investigated whether our MV-Ao intervals had a tendency to change over time by plotting the results of our 207 pregnancies (not including five pregnancies that developed AVB III) by the order they were recruited into the surveillance programme and did not find any reason to suspect such an effect. No trend was observed either by visual examination (data not shown) or by linear regression; MV-Ao time interval (in milliseconds): $y = 129 - 0.006x$, $r = 0.04$, $P = 0.57$; MV-Ao interval (*z*-scores): $y = 1.55 - 0.0007x$, $r = 0.04$, $P = 0.56$. Postnatal ECG was recorded within 1 week after birth. First-degree AVB was defined as a PR interval exceeding the 98th percentile of previously published reference values [19]. In all categorical analyses, AVB II–III was defined as an MV-Ao time interval >3 S.D. or a PR interval exceeding the 98th percentile.

Statistical analysis

Statistical analyses were performed using STATISTICA 12 (StatSoft, Tulsa, OK, USA). Variables with a skewness and kurtosis within ±1.0 were accepted as being normally distributed. Linear regression with *F*-test (maternal age), one-way analysis of variance with planned comparisons (seasons of the year) and *t*-tests were used for continuous data. For variables not fitting a normal distribution (PR interval on ECG), we used the Mann–Whitney *U*-test or Wilcoxon matched pairs test as appropriate. Categorical data were analysed using Pearson χ^2 or Fisher's exact test when any cell had fewer than five observations. We have previously published data from 85 [13] and 186 [20] cases included in the present study.

Results

Fetal and neonatal outcome

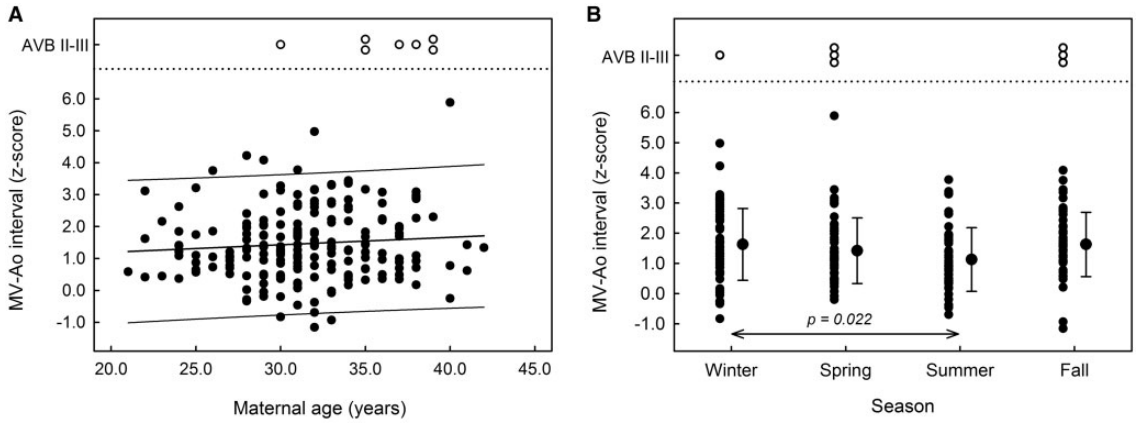
From 2000 to 2015, a total of 212 anti-Ro52/SSA antibody-exposed fetuses were followed by serial Doppler echocardiography during weeks 18–24 of pregnancy and included in this study. Signs of abnormal AV conduction *in utero*, defined as either MV-Ao interval >2 S.D. or MV-Ao interval >3 S.D., were detected in 63 and 29 fetuses, respectively. One of these fetuses died unexpectedly at 32 weeks of gestation, and a second- or third-degree AVB developed in two and five fetuses, respectively. Treatment with betamethasone reverted the block in the two cases of AVB II. After birth, an abnormal ECG was documented in 25 of 184 babies where a postnatal ECG was available, whereof 20 had a first-degree AVB and 5 AVB III. The remaining 28, for whom a postnatal ECG was not available, had a normal heart rate at birth and their routine child care check-up.

The cohort comprised 99 female and 112 male fetuses, and 1 lost to follow-up. We did not observe any effect of gender on AV time intervals measured *in utero* or on ECG outcome at birth (data not shown).

Maternal age, fetal AV time interval and development of AVB

We had previously observed that maternal age was associated with CHB in a population-based study of anti-Ro/SSA antibody-associated CHB [8]. In the present cohort, we did not find any significant correlation between

FIG. 1 Influence of maternal age and season on fetal atrioventricular conduction in anti-Ro52/SSA-positive pregnancies



(**A**) Fetal Doppler MV-Ao time intervals are not correlated with maternal age in 205 cases without signs of AVB II–III ($y = 0.73 + 0.023x$, $S_{y/x} = 1.11$, $r = 0.09$, P = 0.22). A significant difference is, however, found when these cases (filled circles) are compared with those having AVB II–III (open circles; 31.4 ± 4.1 *vs* 36.1 ± 3.2 years, P = 0.003). (**B**) Fetal Doppler AV time categorized by the time of the year recorded. Season of the year was not a significant factor of variance when comparing all four seasons (P = 0.086), but a significant effect was observed when comparing fetuses surveilled during the winter months (December–February) and the summer (June–August), *z*-score 1.63 ± 1.19 *vs* 1.13 ± 1.05, P = 0.022. AV: atrioventricular; MV-Ao: left ventricular in- and outflows.

maternal age and MV-Ao time intervals in fetuses without signs of AVB II–III (Fig. 1A, $y = 0.73 + 0.023x$, $S_{y/x} = 1.11$, $r = 0.09$, P = 0.22). However, maternal age proved to be significantly higher in the seven pregnancies complicated by fetal AVB II–III compared with the 205 pregnancies without AVB II–III also in the present study (36.1 ± 3.2 *vs* 31.4 ± 4.1 years, mean ± SD, P = 0.003).

Season and fetal AV time interval

We had also previously observed that season of birth was significantly associated with CHB development [8], and we therefore investigated whether season had an influence on fetal AV time intervals in the present cohort. In particular, we had found that an increased proportion of CHB cases were born in the summer, raising the hypothesis that events associated with the winter season, which would coincide with the pregnancy period during which CHB develops for babies born in the summer, could influence the risk for CHB. In the present study, we therefore directly compared winter and summer measurements of fetal AV time intervals taken during the CHB risk period of pregnancy. Notably, AV time intervals of fetuses surveilled during the winter months were significantly longer than those of fetuses surveilled during the summer (*z*-scores 1.63 ± 1.19 *vs* 1.13 ± 1.05, P = 0.022; Fig. 1B and Table 1), although the season of year did not appear to be a significant factor of MV-Ao time interval variance when comparing all four seasons (P = 0.086). The proportion of fetuses with AV time intervals >2 SD. was higher in pregnancies with the CHB risk period taking place during the winter *vs* those for which the risk period fell in the summer (37 *vs* 15%, P = 0.014), but no difference was found in the proportions of cases with an abnormal ECG at birth (P = 0.76; Table 1).

Fetal and neonatal outcome in relationship to outcome of previous pregnancy

Recurrence of CHB in autoantibody-positive pregnancies has been found to range between 12 and 20% in several independent studies [8–12]. However, as these studies include second- and mostly third-degree AVB only, it is possible that a greater proportion of pregnancies following a first CHB case show signs of abnormal fetal/neonatal AV conduction, if cases with subtler or more transient AV conduction disturbances are taken into account. Likewise, it is also unknown whether the presence of conduction disturbances in a first pregnancy, including not only severe cases of AVB II–III but also abnormal, prolonged fetal AV time intervals, is associated with the presence of fetal conduction disturbances in a subsequent pregnancy.

In our cohort of 212 anti-Ro/SSA antibody-exposed fetuses, there was a history of AVB II–III in the preceding pregnancy in eight fetuses. One of these eight fetuses developed a 2:1 AVB that converted during betamethasone treatment. Six fetuses out of 204 without an AVB II–III history in previous pregnancies developed AVB II–III during surveillance. Comparison of the eight fetuses with a history of AVB II–III with the rest of the cohort revealed that a history of AVB II–III was associated with significantly longer MV-Ao time intervals (*z*-scores 2.54 ± 0.60 *vs* 1.43 ± 1.11, P = 0.005) and a significantly greater proportion of fetuses showing signs of abnormal AV conduction defined as MV-Ao interval >2 SD. (88 *vs* 27%, P < 0.001; Table 2). A similar trend was observed when using a higher threshold, MV-Ao interval >3 SD., although the

Table 1 Atrioventricular conduction in Ro/SSA-exposed fetuses relates to season

Variable	Winter	Summer	P-value
Fetal Doppler[a]			
Heart rate, beats/min	145 (5.8)	145 (6.5)	0.66
MV-Ao interval, ms	130 (10.6)	126 (9.5)	0.025
MV-Ao interval, *z*-score	1.63 (1.19)	1.13 (1.05)	0.022
MV-Ao interval >2 S.D., n (%)	22/60 (37)	7/46 (15)	0.014
MV-Ao interval >3 S.D., n (%)	9/60 (15)	4/46 (9)	0.38
Post-natal ECG[a]			
Heart rate, beats/min	124 (19.6)	131 (24.6)	0.12
PR interval, ms, median (IQR)	102 (100–110)	100 (98–110)	0.90
PR interval >98th percentile, n (%)	8/57 (14)	4/40 (10)	0.76

[a]Grouped by the season (winter or summer) during which the congenital heart block risk period occurred and fetal Doppler measurements were made. Values are the mean (S.D.) unless otherwise stated. IQR: interquartile range; MV-Ao: left ventricular in- and outflows.

Table 2 Atrioventricular conduction in Ro/SSA-exposed fetuses relates to previous sibling's history of congenital heart block

Variable	History	No history	P-value
Fetal Doppler[a]			
Heart rate, beats/min	141 (6.2)	144 (5.9)	0.11
MV-Ao interval, ms	138 (5.3)	128 (9.9)	0.006
MV-Ao interval, *z*-score	2.54 (0.60)	1.43 (1.11)	0.005
MV-Ao interval >2 S.D., n (%)	7/8 (88)	56/204 (27)	<0.001
MV-Ao interval >3 S.D., n (%)	3/8 (38)	26/204 (13)	0.080
Post-natal ECG[a]			
Heart rate, beats/min	130 (23.1)	128 (20.5)	0.78
PR interval, ms, median (IQR)	120 (104–124)	100 (98–110)	0.035
PR interval >98th percentile, n (%)	4/7 (57)	21/177 (12)	0.007

[a]Grouped by history of atrioventricular block II–III in previous siblings. Values are the mean (S.D.) unless otherwise stated. IQR: interquartile range; MV-Ao: left ventricular in- and outflows.

difference was no longer significant (38 *vs* 13%, P = 0.080). Importantly, we also found that a history of AVB II–III was associated with significantly longer PR intervals (P = 0.035) and a higher proportion of cases with abnormal AV conduction at birth (57 *vs* 12%, P = 0.007) on post-natal ECG (Table 2).

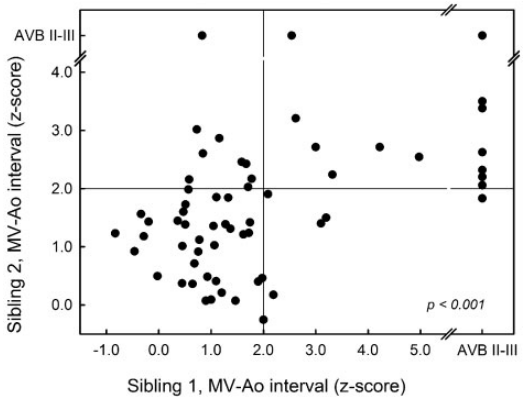
Fifty-seven women were included in the surveillance programme for two consecutive pregnancies. Another six were included in the programme after a previous pregnancy with fetal AVB II–III diagnosed at our unit. These 63 mothers allowed us to investigate directly the relationship between fetal AV conduction in two consecutive pregnancies (siblings 1 *vs* 2). However, this restricted group of cases also included four of our seven fetuses that developed AVB II–III during surveillance, reducing the number of cases in the statistics using MV-Ao time intervals and PR intervals as continuous variables to 53 pairs. We observed no difference in time intervals measured *in utero* or at birth between siblings 1 and 2, indicating no obvious trend of increasing conduction time with increasing parity (Table 3). Interestingly however, we found that MV-Ao time intervals recorded during consecutive pregnancies were significantly correlated, with abnormal fetal AV conduction during the first pregnancy being associated with a longer MV-Ao time interval in the next pregnancy (P < 0.001; Fig. 2). In addition, the presence of AV conduction disturbances during mid-trimester of the first pregnancy, defined as exceeding +2 or +3 S.D., was associated with significantly longer MV-Ao time intervals *in utero* and PR intervals at birth, as well as a significantly greater proportion of cases with abnormal time intervals before and after birth, in the subsequent pregnancy (Table 4). Notably, 38% of babies following a previous sibling with fetal AVB II–III or an MV-Ao time interval >2 S.D. had a PR interval exceeding the 98th percentile in ECG after birth. For babies with an MV-Ao time interval >3 S.D., the corresponding number was even higher; 45%. We could also demonstrate that mothers with an MV-Ao time interval >2 S.D. in sibling 1 (excluding AVB II–III cases) had a higher proportion of fetuses with

Table 3 Atrioventricular conduction in 106 siblings followed during two successive singleton pregnancies in 53 anti-Ro52/SSA-positive women

Variable	Sibling 1	Sibling 2	P-value
Fetal Doppler			
Heart rate, beats/min	145 (5.9)	144 (6.0)	0.31
MV-Ao interval, ms	127 (10.3)	128 (8.0)	0.58
MV-Ao interval, *z*-score	1.27 (1.14)	1.35 (0.91)	0.61
Post-natal ECG			
Heart rate, beats/min	129 (23.9)	124 (18.7)	0.35
PR interval, ms, median (IQR)	100 (94–110)	100 (98–110)	0.93

Values are the mean (S.D.) unless otherwise stated. IQR: interquartile range; MV-Ao: left ventricular in- and outflows.

Fig. 2 Correlation between fetal atrioventricular conduction in two consecutive pregnancies in 63 anti-Ro52/SSA antibody-positive women



Each pair of siblings is presented as a filled circle with a position defined by the Doppler-derived atrioventricular conduction in sibling 1 on the *x*-axis and sibling 2 on the *y*-axis. Continuous lines denote an MV-Ao time interval of + 2 S.D. Abnormal AV conduction (MV-Ao interval >2 S.D. or AVB II–III) in sibling 1 was significantly associated with abnormal AV conduction in sibling 2 ($\chi^2 = 15.4$, $P < 0.001$). AV: atrioventricular; AVB: atrioventricular block; MV-Ao: left ventricular in- and outflows.

time interval >2 S.D. also in sibling 2 (6/10 *vs* 9/47, P = 0.018). As our number of cases with more severe prolongation of AV time intervals was small, we have refrained from analysing the cases with AVB II–III and prolonged AV time intervals separately.

Altogether, our data indicate that the recurrence rate of fetal conduction disturbances in women with anti-Ro/SSA antibodies is substantially higher than what has previously been found for AVB II–III when milder forms of cardiac conduction disturbances are taken into account.

Discussion

To date, only the presence of maternal anti-Ro/SSA autoantibodies and a history of fetal CHB are well-recognized risk factors for CHB. However, given that CHB develops in 1–2 and 12–20% of pregnancies with these respective risk factors, the identification of additional factors influencing CHB occurrence remains an important goal, not only to improve our understanding of CHB pathophysiology, but also for better identification of pregnancies at risk to allow for close surveillance and immediate therapeutic intervention. In this study, we used data gathered from 212 anti-Ro52/SSA antibody-positive pregnancies prospectively followed by serial Doppler echocardiography at a single centre to assess whether the association of maternal age and season of birth with CHB, which we had previously shown in a population-based study of CHB, could be confirmed, and if these factors would also impact AV time intervals measured *in utero*. We also investigated the recurrence of fetal conduction disturbances, including both milder and more severe forms of CHB.

First, we found that the maternal age in pregnancies complicated by fetal AVB II–III was significantly higher than that in pregnancies without signs of fetal AVB II–III, confirming our previous observation [8]. However, we did not find any significant correlation between maternal age and Doppler AV time intervals in non-AVB II–III pregnancies. Other studies of maternal age in relationship to development of CHB have not found any association [16, 21], and the question of whether maternal age is a significant risk factor for CHB or not remains. Our present observation that maternal age is associated with AVB II–III but not fetal AV time interval might suggest that the main impact of age-related factors is on the inflammatory processes leading to fetal AV node fibrosis and AVB II–III occurrence rather than on the occurrence of transient/mild conduction disturbances. Little is known about placental function in relationship to maternal age, but a suboptimal placental function or a higher propensity to produce pro-inflammatory cytokines might contribute to complete CHB. Another possible risk factor for fetal AVB previously described and related to age might be the presence of hypothyroidism [22].

Second, we observed that the Doppler AV time intervals measured *in utero* during the CHB risk period were significantly longer in the winter compared with summer. These findings are in line with our previous observation

Table 4 Atrioventricular conduction in 63 offspring of the second of two successive pregnancies

Sibling 2	Sibling 1 fetal Doppler MV-Ao interval					
	>2 s.d.	<2 s.d.	P-value	>3 s.d.	<3 s.d.	P-value
Fetal Doppler[a]						
Heart rate, beats/min	142 (6.8)	144 (5.8)	0.28	143 (6.6)	144 (6.0)	0.19
MV-Ao interval, ms	136 (7.3)	126 (7.5)	<0.001	136 (5.7)	127 (8.0)	<0.001
MV-Ao interval (*z*-score)	2.26 (0.83)	1.21 (0.85)	<0.001	2.35 (0.64)	1.27 (0.91)	<0.001
MV-Ao interval >2 s.d., n (%)	13/18 (72)	9/45 (20)	<0.001	10/13 (77)	12/50 (24)	<0.001
Post-natal ECG[a]						
Heart rate, beats/min	125 (19.3)	127 (19.6)	0.76	130 (18.9)	125 (19.6)	0.48
PR interval, ms, median (IQR)	110 (100–120)	100 (96–105)	0.003	115 (106–124)	100 (97–106)	0.002
PR interval >98th percentile, n (%)	6/16 (38)	2/38 (5)	0.006	5/11 (45)	3/43 (7)	0.006

[a]Grouped in accordance with the observations made during the first pregnancy. Values are the mean (s.d.) unless otherwise stated. IQR: interquartile range; MV-Ao: left ventricular in- and outflows.

that there is a greater proportion of CHB cases born in the summer, which coincides with the CHB risk period falling in the winter [8] and supports the hypothesis that events associated with the winter season may affect the development of fetal conduction disturbances in anti-Ro/SSA antibody-positive pregnancies. Of note, in the present study, no significant difference was found in the presence of conduction disturbances at birth between pregnancies with the CHB risk period falling in the winter *vs* summer. This may perhaps be explained by the fact that a substantial proportion of prolonged fetal AV time intervals normalize before birth [13] and that the present study, based on a single-centre experience, includes few cases of AVB II–III, altogether reducing the number of events observed at birth and decreasing the power to detect any potentially significant effect of season on AVB occurrence at birth. However, it might also suggest that events associated with the winter season predominantly impact the performance of the fetal cardiac cells and the AV conduction time without necessarily inducing long-lasting effects or being sufficient to lead to complete AVB.

Third, several studies have established that the recurrence rate for (complete) CHB ranges from 12 to 20%, indicating that maternal anti-Ro/SSA antibodies are not sufficient to induce CHB [8–12]. In our cohort, when taking into account milder manifestations of cardiac conduction disturbances, such as abnormal, prolonged fetal AV time intervals, we found recurrence rates higher than those observed for AVB II–III. Specifically, AVB I was present at birth in 57% of babies born after a sibling with AVB II–III. In addition, fetal AV time intervals in consecutive pregnancies were significantly correlated, and AVB I–III was observed at birth in 38% of babies born after a sibling with evidence of abnormal fetal AV conduction.

Collectively, these findings indicate that maternal autoantibodies may impact the fetal heart more consistently than previously thought, and that the mothers may be referable to two distinct groups: one for which the risk of fetal conduction abnormalities is high, and one for which the risk is very low. Interestingly, this is in line with the suggested two-step hypothesis for the development of CHB, which suggests that, if the right antibody specificity is present, maternal anti-Ro/SSA antibodies would consistently affect the fetal heart cells' conduction performance; additional factors would then be required both for detectable conduction disturbances to occur and for complete AVB to develop. The fact that we did not always detect conduction disturbances, even mild, in a pregnancy following an affected one supports this idea that additional factors are necessary for detectable conduction disturbances to develop, even in the presence of pathogenic maternal antibodies. Events associated with the winter season, as discussed above, might be one such factor modulating the penetrance and/or magnitude of conduction disturbances induced by maternal autoantibodies, for example, by affecting maternal autoantibody titres. Fetal genetics, influencing how well the fetal heart cells may cope with the burden imposed by maternal autoantibodies, is also a likely candidate [23]. Finally, considering that the recurrence rate of AVB II–III is lower than the recurrence rate we observed in our cohort when including milder conduction disturbances, it is likely that different and/or additional risk factors are needed for AVB II–III to develop compared with milder conduction disturbances. In particular, it is likely that additional factors promoting full-blown inflammation in the fetal heart are needed for the establishment of complete AVB; here, fetal genetics influencing the fetal immune response and fibrosis of the AV node are likely candidates [24–27].

In conclusion, our findings paint a complex picture of CHB development in anti-Ro/SSA antibody-positive pregnancies, with multiple factors being involved and impacting the phases of CHB development differently. In particular, our observations reveal that the recurrence

of fetal conduction disturbances in fetuses of women with anti-Ro/SSA antibodies is higher than previously reported when milder forms of conduction disturbances are taken into account, pointing to a more consistent effect of maternal autoantibodies than what was previously thought. Additional risk factors related to maternal age and season, as suggested by our study, as well as other environmental and genetic factors, would then modulate the impact of maternal autoantibodies and contribute to CHB development. Ideally, such factors should be explored in more detail in prospective studies to increase our understanding of CHB aetiopathogenesis, clearly identify pregnancies at risk and improve therapeutic options.

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