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Postprint available at: Linköping University Electronic Press http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-75459 Development of heart block in SSA/SSB autoantibody-positive pregnancies is associated with maternal age and display a season-of-birth pattern

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ABSTRACT

Objective. Congenital heart block may develop in the fetus of Ro/SSA and La/SSB positive mothers. Reported recurrence rates of only 10-20% despite persisting maternal antibodies indicate that additional factors are critical for establishment of the block. We therefore investigated the influence of other maternal and fetal factors on heart block development in a Swedish population-based cohort.

Methods. The influence of fetal gender, maternal age, parity and time of birth on heart block development was analyzed in 145 families including Ro/La-positive (n= 190) and Ro/La-negative (n=165) pregnancies.

Results. We observed a recurrence rate of 12.1% in Ro/La-positive women, and no recurrence in Ro/La-negative women. Fetal gender and parity did not influence the development of heart block in either group. Maternal age in Ro/La-positive pregnancies with a child affected by heart block was however significantly higher than in pregnancies resulting in babies without heart block (p<0.01). Further, seasonal timing of pregnancy influenced the outcome. Gestational susceptibility-weeks 18-24 occurring during January-March correlated with a higher proportion of heart block pregnancies and lower vitamin D levels, and a corresponding higher proportion of children with heart block born in the summer (p<0.02). Maternal age or seasonal timing of pregnancy did not affect the outcome in Ro/La-negative pregnancies.

Conclusion. This study identifies maternal age and seasonal timing of pregnancy as novel risk factors for congenital heart block development in Ro/La positive pregnancy. These observations indicate that the risk can be modified, and will be important for counseling when a pregnancy is considered.

Congenital complete heart block without cardiac malformation is a rare disease, affecting 1 in 15,000 to 20,000 births in the general population. An association with the presence of maternal autoantibodies to Ro/SSA and/or La/SSB is however well established [1, 2], and the risk of complete congenital heart block is 1-2% in Ro/SSA-positive pregnancies [3-6]. Furthermore, the reported risk of giving birth to a second child with complete heart block for anti-Ro/SSA positive mothers ranges from 12 to 20% [7-9], despite the persistence of the maternal autoantibodies [10]. This indicates that additional factors are critical for establishing the heart block. Fetal genetic susceptibility has been suggested as a potential risk factor [11, 12], and polymorphisms in the gene encoding TGF β have been implicated in the development of heart block [13, 14]. Variations in the intrauterine environment between pregnancies have also been suggested to contribute to the penetrance of the disease. Maternal disease severity has been investigated as such a potential risk factor, but was not found to contribute to the development of congenital heart block [15].

Given the rarity of congenital heart block occurrence in the general population, it is difficult to investigate potential risk factors associated with the disease. In particular, very little information is available on the influence of maternal age and parity on pregnancy outcome in anti-Ro/La positive mothers. In an effort to address these questions in a reasonable cohort we identified heart block patients in the Swedish population via national health care and hospital registries and investigated maternal and fetal factors associated with congenital heart block in one hundred and forty-five families. Variables analyzed included maternal serological status, age, parity as well as fetal gender and season of birth.

PATIENTS AND METHODS

Patients and families

Identification and enrollment of patients were described in detail previously [16]. Briefly, patients with heart block diagnosed before 15 years of age were identified using the Swedish National Patient Register (www.socialstyrelsen.se) and the Swedish Pacemaker and ICD Register (www.pacemakerregistret.se), as well as by local clinical registers and through a network of pediatric and adult cardiologists and rheumatologists at the six university hospitals in Sweden. Patients with cardiac structural abnormalities as well as patients with post-operative or infection-induced block were excluded from the study. Patients and their families were contacted and offered to take part in the study and donate blood samples. Families for whom a) a blood sample from the mother, b) information on date of birth for the mother and all siblings were available were included in the present study. In total, 145 families including 149 individuals with heart block born between 1949 and 2009, 145 mothers and 208 siblings without heart block were included in the study. The study was approved by the Regional Ethical Committee, Stockholm, Karolinska Institutet, and all participants, or the parents if the individual was <18 years, gave informed written consent.

Serologic analyses

Autoantibodies were detected by Inno-LiaTM ANA (Innogenetics, Cambereley, United Kingdom) according to the manufacturer's instructions and antibodies to Ro52 further confirmed by ELISA [17].

Vitamin D levels

For evaluation of vitamin D levels, data were obtained from female Swedish individuals (n=1068), selected as controls from the national population register as described [18]. Vitamin D levels were measured as serum 25(OH) vitamin D by a chemiluminescent assay which recognizes 100% of both 25(OH) D₂ and 25 (OH) D₃) (Liason ®, DiaSorin, Saluggia, Italy).

Statistical analyses

Statistical analyses for fetal gender and time of birth were performed using a Chi-square test. Difference in maternal age between antibody-positive or -negative pregnancies leading to the birth of a child with or without heart block was evaluated by Kruskal-Wallis test followed by Dunn's post test. Difference in maternal age at the birth of the first child was evaluated by Mann-Whitney U-test. The odds ratios (OR) of developing heart block associated with each maternal age category, with and without adjustment for parity, together with 95% confidence intervals, were calculated by logistic regression. The correlation between vitamin D levels for each month of the year and the ratio between the numbers of AVB or healthy pregnancies for which the 21^{st} week fell in that particular month was assessed by the non-parametric Spearman correlation test. A p value < 0.05 was considered significant. Statistical analyses were performed using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, California, USA (www.graphpad.com), as well as Statistica 8.0, Statsoft, Tulsa, OK and SAS software for Windows version V.9.1.

RESULTS

Distinction of families with or without maternal autoantibodies

One hundred and forty-five families with at least one child diagnosed with heart block before the age of fifteen were included in the study. In 80 families (55%) the mother carried autoantibodies to Ro and/or La and in 65 families (45%) the mother had no detectable anti-Ro/La autoantibodies [16]. The presence versus absence of maternal antibodies has been suggested to define two distinct groups of heart block patients regarding pathogenic mechanisms [19], and we therefore investigated the maternal and fetal factors potentially associated with heart block separately in Ro/La-positive (n=190) and Ro/La-negative (n=165) pregnancies.

Recurrence of heart block in subsequent pregnancies

Of the 80 families enrolled in the study where the mother tested positive for anti-Ro/La antibodies, 33 (41%) had additional children after the birth of a child with heart block. Of the 33 pregnancies immediately following the birth of the first affected child, 4 resulted in the birth of another child with heart block, generating a recurrence rate of 12.1% (Figure 1). The overall familial recurrence rate was 8.9%, with 4 affected pregnancies out of a total of 45 including all pregnancies occurring after the birth of a child with heart block.

Of the 65 families where the mother was negative for the presence of anti-Ro/La antibodies, 36 (55%) included one or more siblings born after a child with heart block. However, none of the pregnancies following the birth of the first heart block case was affected, generating a recurrence rate of 0% in this group (Figure 1).

Fetal gender of heart block patients versus healthy siblings

The influence of fetal gender on the development of heart block was next investigated. Female sex was observed in 52% of the affected children and 56% of the healthy children born to anti-Ro/La positive mothers, indicating that fetal sex did not predict occurrence of heart block (p = 0.661). Similarly, no significant skewing of the gender distribution was observed in the group of children born to anti-Ro/La negative mothers, with 57% of affected and 53% of healthy children being female (p = 0.580), Table 1.

Maternal age, but not parity, influences pregnancy outcome in Ro/La-positive mothers

While the risk of pregnancy complications in general increases with the age of the mother, little is known about the influence of maternal age or parity on pregnancy outcome and heart block development in Ro/La positive women. We therefore investigated the influence of these maternal factors on the development of heart block.

We first analyzed if there was any difference in the age of the mothers in the autoantibody-positive compared to the autoantibody-negative group. The age of the mother at the birth of a child with heart block was significantly higher in anti-Ro/La positive women than in antibody-negative women, 29.5 (28.4-30.6) years; mean (95% confidence interval), versus 26.9 (25.8-27.9) years respectively, p < 0.01 (Figure 2A). Since women with anti-Ro/La antibodies represent a group where the prevalence of autoimmune diseases such as Sjögren's syndrome and systemic lupus erythematosus will be much higher than in the group of anti-Ro/La negative women, it is possible that the observed difference in maternal age results from a less favorable health status of antibody-positive women and a delayed maternity in general. However, we did not observe any significant difference in the maternal age at the birth of the first child between antibody-positive and -negative women (p=0.069, Mann-Whitney U test), Figure 2B.

We next compared the age of the mothers in Ro/La antibody-positive pregnancies when giving birth to a child with, or without, congenital heart block. Anti-Ro/La positive mothers were significantly older at the time of a pregnancy resulting in a child affected with heart block than at the time of birth of a non-affected baby; 29.5 (28.4-30.6) years, mean (95% confidence interval), versus 27.5 (26.5-28.5) years respectively, p < 0.05 (Figure 2A). An obvious correlate of increasing age is increasing parity, and to clarify the effect of parity on heart block development we analyzed the risk of having a child with heart block in relation to parity and maternal age. Stratifying the different pregnancies by parity, we observed that anti-Ro/La positive mothers giving birth to a child with heart block were consistently older than antibody-positive women giving birth to a healthy child, independently of whether it was the first, second, third or higher number of pregnancy (Figure 2C and 2D). This trend was not observed in the group of antibodynegative mothers (Figure 2D). Analysis of the influence of maternal age and parity on the risk of having a child with heart block in antibody-positive women revealed that only maternal age had a significant effect (p = 0.01) (logistic regression model analysis), while parity did not significantly affect pregnancy outcome (p = 0.35).

To estimate the increase in risk due to increasing maternal age, we calculated the odds ratio (OR) for giving birth to a child with heart block in anti-Ro/La antibody-positive women in relation to age (Figure 2E and Table 2). Increasing OR with increasing maternal age was observed, with OR 2.3 (1.0-5.5, 95% confidence interval, p = 0.05) and OR 4.2 (1.4-11.9, p = 0.01) for women 30-34 and \geq 35 years old, respectively, compared to women <25 years of age. In addition, we did not find any evidence of an interaction between maternal age and parity regarding increased odds ratio for the risk of heart block (p = 0.21). No significant influence of maternal age or parity was observed in the group of anti-Ro/La antibody negative women (Table 2).

Season-of-birth influence development of congenital heart block

Evidence that the time of birth as defined by season or month may influence the development of autoimmune diseases later in life has lately emerged [20]. Heart block in anti-Ro/La-positive pregnancies usually develops between 18 to 24 weeks of pregnancy, indicating that the pathogenic mechanisms leading to conduction defects in the fetal heart are initiated before and/or within this period of pregnancy. The 18-24 weeks of pregnancy taking place during the late Swedish winter months (January-March) corresponds to a birth occurring during the Swedish summer (June-August), and we hypothesized that events linked to the winter season may affect the outcome of pregnancy and the ratio of healthy and affected births during the summer as compared to the rest of the year.

Analysis of the number of births during the summer (June-August) and the rest of the year revealed a significant difference between affected children and healthy siblings in the Ro/La-positive pregnancy group, with births of children affected by heart block representing 58.5% of all births in the summer and only 39.0% of all births during the rest of the year (p = 0.015, Figure 3A). This skewed distribution towards an increase in the proportion of affected births in the summer was not observed in the Ro/La-negative pregnancy group (p = 0.583, Figure 3B).

The significant increase in the proportion of children with heart block born in the summer as compared to the rest of the year supported our hypothesis that events linked to the winter season and occurring during the susceptibility weeks 18-24 may affect the outcome of the pregnancy. Such a possible factor is the marked decrease in light exposure during winter in Nordic countries like Sweden that may affect vitamin D levels. We therefore analyzed the variation of vitamin D levels over the year based on samples from 1068 Swedish women that had

been taken at different times of the year. Vitamin D levels were higher during the summer, with the highest mean value observed in August, than during the winter months, with the lowest mean level recorded in March (Figure 3C). We found that average vitamin D levels for each month were significantly correlated to the ratio of heart block to healthy pregnancies for which the median susceptibility week (defined as week 21 of pregnancy) fell in that particular month (p = 0.009), with low vitamin D levels corresponding to a significantly higher proportion of heart block pregnancies (Figure 3D).

DISCUSSION

Isolated complete congenital heart block is well known to associate with the presence of maternal anti-Ro/SSA and La/SSB antibodies. A recurrence rate of 12-20% however indicates that additional factors are involved in the pathogenic process. The objective of the present study was to investigate the influence of variables such as maternal age, parity, time of birth and fetal gender in the development of heart block. More specifically, we assessed the potential association of these factors with congenital heart block not only by comparing heart block cases to healthy siblings in a cohort of families with autoantibody-positive mothers, but also by analyzing the same variables in a group of heart block patients and healthy siblings born to autoantibody-negative mothers.

In the anti-Ro/La positive group of mothers we observed a recurrence rate of 12.1% in 33 pregnancies immediately following the birth of a child with heart block, and an overall recurrence rate in all siblings born after an affected child of 8.9%. While low recurrence rates have already been reported [21], recurrence rates of 17% in pregnancies immediately following the birth of a child with congenital heart block have been observed in large cohorts in two independent studies [8, 22]. However, recurrent cases in both of these studies may be overrepresented as the cohorts were formed either by specifically recruiting families with cases of neonatal lupus [22] or based on registers from tertiary referral centers [8]. By contrast, in this study we analyzed a population-based cohort gathered from national health registers in an effort to minimize the bias towards recruitment of families with multiple heart block cases.

Fetal gender was not associated with development of heart block in Ro/La-positive or negative pregnancies, in accordance with previous reports [9, 22]. A trend towards higher maternal age for heart block mothers compared to mothers who give birth to healthy babies was

previously reported by us [23]. In the present study we found that the risk of fetal heart block in Ro/La-positive pregnancies increased significantly with increasing maternal age but was not influenced by parity. This finding was specific for autoantibody-associated heart block, as neither maternal age nor parity influenced the outcome of Ro/La-negative pregnancies.

Complications during pregnancy have been associated with increasing maternal age, affecting both the mother and the child, reviewed in [24]. Disorders such as placental growth retardation and placental abruption are known to increase in frequency with age, mainly due to insufficient utero-placental perfusion. However, there is little information about placental function in relation to maternal age. This factor may be relevant in the context of congenital heart block as hypoxia has been suggested to amplify the harmful effects of anti-Ro/La antibodies in the fetal heart [25]. Hypothyroidism, known to be associated with aging, may also be another factor of interest as anti-Ro positive women with hypothyroidism have been shown to be at higher risk of having a child with complete congenital heart block compared to women with autoantibodies alone [26]. It is also possible that the increasing risk for heart block with increasing maternal age reflects the appearance of anti-Ro/La autoantibodies in women over time. This hypothesis could however not be addressed in the present study, as blood samples from mothers were only collected after the birth of a child with heart block.

Seasonal variation in the time of birth has recently been associated with the development of autoimmune diseases later in life, eg multiple sclerosis [20]. We speculated that events such as low wintertime vitamin D levels at the critical period of potential heart block development in fetuses of women with anti-Ro/La autoantibodies might affect the outcome of the pregnancy. This specific time window (18-24 weeks of gestation) during the winter corresponds to birth in the summer. In the present study we found that the distribution of heart block births over the year was skewed towards an increased proportion of affected children born in the summer.

Additionally, we found that vitamin D levels, which varied in a Swedish female population over the year in relation to the seasons, were inversely correlated to the number of heart block pregnancies in autoantibody-positive women. Although the vitamin D levels used in this study originated from a population of healthy Swedish women and not from the specific mothers of our cohort, it is reasonable to believe that these women would follow the same general trend. Despite the well-documented immunomodulatory role of vitamin D (reviewed in [27]), its potential relation to the development of heart block *in utero* remains to be elucidated. While the association between the winter season, decreased sun exposure and vitamin D levels comes readily to mind, other events linked to the winter season such as viral infections might also influence the development of heart block.

In summary, we report a recurrence rate of 12.1% in anti-Ro/La positive pregnancies and demonstrate a significant association of increased maternal age and birth in the summer with development of heart block in antibody-positive pregnancies. These associations were not observed in antibody-negative pregnancies. Our data support the idea of different pathogenic mechanisms of heart block in anti-Ro/La positive and negative pregnancies, and are also of great interest to improve the counseling of antibody-positive women contemplating pregnancy.

 Table 1. Association between fetal gender and pregnancy outcome.

	Total n	Female	Male n (%)	p *
Ro/La+ pregnancies				
AVB	84	44 (52)	40 (48)	0.661
Healthy	108	60 (56)	48 (44)	
Ro/La- pregnancies				
AVB	65	37 (57)	28 (43)	0.580
Healthy	99	52 (53)	47 (47)	

^{*} p values were determined by the Chi-square test.

Table 2. Odds ratio of fetal heart block in relation to maternal age.

	Age	OR (95% CI)	p	OR (95% CI)	p
			-	Adjusted for parity	
Ro/La+ pregnancies	≤ 24	1.0 (Reference)	Reference	1.0 (Reference)	Reference
	25-29	1.7 (0.8-3.7)	0.18	1.6 (0.7-3.6)	0.22
	30-34	2.0 (0.9-4.5)	0.09	2.3 (1.0-5.5)	0.05
	≥ 35	3.2 (1.2-8.4)	0.02	4.2 (1.4-11.9)	0.01
Ro/La- pregnancies	≤ 24	1.0 (Reference)	Reference	1.0 (Reference)	Reference
	25-29	0.8 (0.4-1.6)	0.46	0.9 (0.4-1.9)	0.77
	30-34	1.1 (0.4-2.6)	0.90	1.7 (0.6-4.9)	0.30
	≥ 35	0.4 (0.1-1.6)	0.20	0.9 (0.2-4.4)	0.90

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COMPETING INTERESTS

None.

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FIGURE LEGENDS

Figure 1. Family size and birth order of heart block cases. Children with heart block are represented by solid black circles, children without heart block are represented by open circles. A solid grey circle represents twins without heart block.

Figure 2. Development of heart block in Ro/La-positive pregnancies is associated with increased maternal age but independent of parity. A. Maternal age at the birth of a child with or without AVB in anti-Ro/La positive or negative pregnancies. B. Maternal age at the birth of the first child for antibody-positive and -negative women. (A,B) Data shown as box plots (25th to 75th percentiles) with line at median and whiskers at minimum and maximum. C. Age of antibody-positive women at the birth of their children, grouped by parity (first to sixth child) and outcome (AVB or no AVB). Line at mean. D. Maternal age at the birth of a child depending on antibody status (anti-Ro/La positive or negative) and pregnancy outcome (AVB or no AVB) presented as the mean with its 95% confidence interval. E. Graphic representation of odds ratio (OR) with 95% confidence interval for giving birth to a child with heart block in autoantibody-positive women dependent on maternal age and adjusted for parity. The reference group corresponds to maternal age ≤24 years (n=16 AVB, 35 no AVB); 25-29 years n=28 AVB, 36 no AVB; 30-34 years n=24 AVB, 26 no AVB; ≥35 years n=16 AVB, 11 no AVB. * p < 0.05, ** p < 0.01.

Figure 3. Development of heart block shows a season-of-birth pattern and correlates to vitamin D levels during the susceptibility weeks 18-24 of pregnancy. A,B. Proportions of affected (grey fields) and healthy (white fields) births during summer and the rest of the year for

anti-Ro/La positive (A) and anti-Ro/La-negative (B) mothers represented as percentage of the total number of births during the respective periods. The number (n) of births in each group is indicated. C. Vitamin D levels (mean, SEM) in a group of healthy Swedish women (n=1068), pooled by month according to blood sampling time. D. Correlation between the average vitamin D levels for each month (mean±SEM) and the ratio of the number of AVB to the number of healthy pregnancies for which week 21 of pregnancy falls in that particular month. *p*-value calculated by the Spearman correlation test.

	Ro/La -	+ mothers	Ro/La - mothers		
Family size, birth- order case pattern	No. of families of each size	No. of families exhibiting case pattern	No. of families of each size	No. of families exhibiting case pattern	
1-child	11		6		
•		11		6	
2-child	36		30		
• 0		9		13	
○ ●		24		17	
• •		3		0	
3-child	27		19		
• 0 0		6		9	
0 • 0		8		5	
• •		1			
○ ●		1		0	
00 •		10		5	
$\circ \bullet \bullet$		1		0	
4-child	4		9		
• 0 0 0		0		2	
0 • 0 0		1		3	
00•0		2		3	
000		1		1	
6-child	2		1		
• 0 0 0 0 0		1		0	
0 • 0 0 0 0		0		1	
000000		1		0	

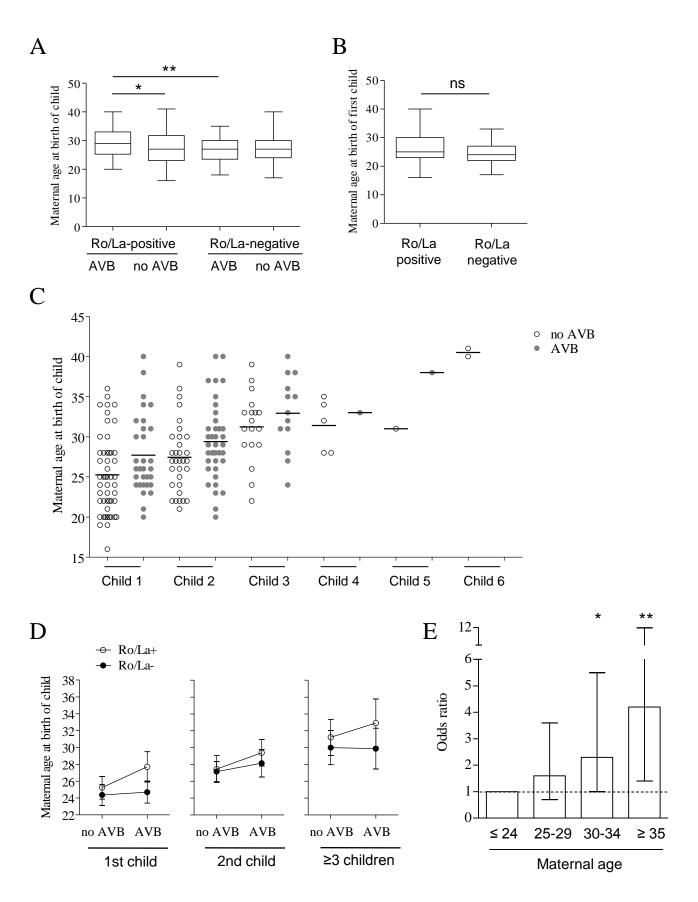


Figure 2, Ambrosi et al

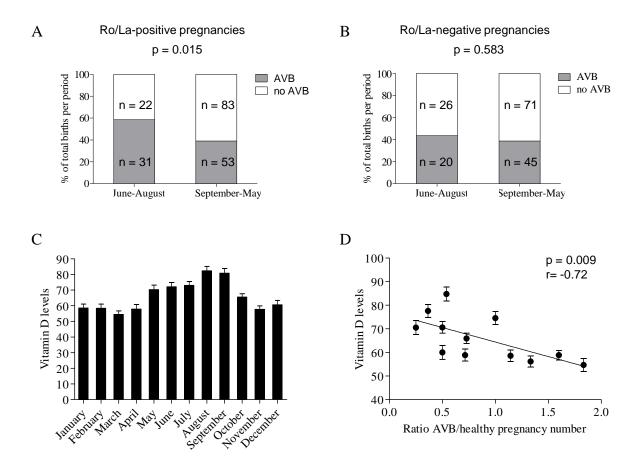


Figure 3, Ambrosi et al