Paper I

Journal of Reproductive Immunology 113 (2016) 27–34

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Maternal anti-HLA class I antibodies are associated with reduced birth weight in thrombocytopenic neonates

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ARTICLE INFO

Article history:
Received 9 June 2015
Received in revised form 16 August 2015
Accepted 19 October 2015

Keywords:
Neonatal thrombocytopenia
Birth weight
Antibodies
Alloimmunization
Placenta

ABSTRACT

In this comparative cross-sectional study, possible associations between maternal anti-HLA class I antibodies and birth weight in neonatal thrombocytopenia are explored. Although commonly detected in pregnancies and generally regarded as harmless, it has been suggested that such antibodies might be associated with fetal and neonatal alloimmune thrombocytopenia (FNAIT). As a link between FNAIT due to human platelet antigen 1a-specific antibodies and reduced birth weight in boys has previously been demonstrated, we wanted to explore whether maternal anti-HLA class I antibodies might also affect birth weight. To examine this, suspected cases of FNAIT referred to the Norwegian National Unit for Platelet Immunology during the period 1998–2009 were identified. Pregnancies where the only finding was maternal anti-HLA class I antibodies were included. An unselected group of pregnant women participating in a prospective study investigating maternal–fetal hemodynamics at the University Hospital North Norway during the years 2006–2010 served as controls. Twenty-nine percent of controls had anti-HLA class I antibodies. The thrombocytopenic neonates had a significantly lower adjusted birth weight (linear regression, $P = 0.036$) and significantly higher odds of being small for gestational age (OR = 6.72, $P < 0.001$) compared with controls. Increasing anti-HLA class I antibody levels in the mother were significantly associated with lower birth weight and placental weight among thrombocytopenic neonates, but not among controls. These results indicate that maternal anti-HLA class I antibodies in thrombocytopenic neonates are associated with reduced fetal growth. Further studies are needed to test if placental function is affected.

1. Introduction

How a semi-allogenic fetus manages to survive pregnancy is still quite an enigma. It is, however, clear that the maternal immune system recognizes and responds to fetal antigens (Arck and Hecher, 2013).

The human leukocyte antigen class I (HLA class I) antigens are present on all nucleated cells and platelets in the human body. The genes that encode HLA class I antigens are the most polymorphic in the human genome. Exposure to incompatible HLA antigens can activate the host immune system and lead to the production of alloantibodies. It is well known that anti-HLA class I antibodies can have severe clinical consequences, such as the rejection of allografts (Lee et al., 2002; Zhang et al., 2005) or the destruction of transfused platelets (Novotny, 1999).

Maternal anti-HLA class I antibodies are commonly detected during pregnancy (approximately 30% of pregnant women) (Morin-Papunen et al., 1984; Regan et al., 1991; King et al., 1996; Masson et al., 2013). In the context of pregnancy, these antibodies are generally considered harmless. Reports have described an association between maternal anti-HLA class I antibodies and recurrent miscarriage (Sargent et al., 1988; Nielsen et al., 2010). Possible associations between maternal anti-HLA class I antibodies and placental abruption (Steinborn et al., 2004) and preeclampsia (Buurma et al., 2012) have also been suggested. However, there are few systematic studies on anti-HLA class I antibodies and pregnancy complications (Lashley et al., 2013).

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http://dx.doi.org/10.1016/j.jri.2015.10.003
0165-0378/

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by maternal alloantibodies targeting human platelet antigens (HPAs) on fetal platelets, most commonly HPA-1a (Davoren et al., 2004; Skogen et al., 2010). FNAIT occurs at a frequency of about 1.5 per 1000 births (Dreyfus et al., 1997; Kjeldsen-Kragh et al., 2007). Intracranial hemorrhage (ICH) is the most severe complication and is reported in around 10% of patients with severe FNAIT (Mueller-Eckhardt et al., 1989; Kamphuis et al., 2010). Numerous reports describe suspected cases of FNAIT with maternal anti-HLA class I antibodies as the only finding and a possible explanation of neonatal thrombocytopenia (Saito et al., 2003; Moncharmont et al., 2004; Thude et al., 2006; Gramatges et al., 2009; Starcevic et al., 2010). It has therefore been suggested that maternal anti-HLA class I antibodies might cause FNAIT.

We have previously demonstrated an association between maternal antibodies against HPA-1a and reduced birth weight in boys (Tiller et al., 2012). The aim of this study was to explore whether there are similar associations between maternal anti-HLA class I antibodies and birth weight in relation to neonatal thrombocytopenia.

2. Methods

2.1. Study population

The two study groups (cases and controls) were identified and selected from pregnant populations that were originally either clinical referrals to the Norwegian National Unit for Platelet Immunology or participants in a different study (Flo et al., 2010, 2014). We performed a secondary analysis of these data using a comparative cross-sectional study design. Selection of the study population is presented as a flow chart in Fig. 1.

All pregnancies referred to the Norwegian National Unit for Platelet Immunology in Tromsø, Norway, for suspected FNAIT during the period 1998–2009 were identified. Pregnancies were included as cases if maternal anti-HLA class I antibodies were detected and neonatal thrombocytopenia was confirmed. Pregnancies were excluded if platelet-specific (anti-HPA-) antibodies were detected or if other reasons for neonatal thrombocytopenia were found. Information regarding demographic characteristics, obstetric history, course, and outcome of pregnancy was obtained from the medical records. All maternal blood samples were taken postpartum.

Of 82 mothers who fulfilled the inclusion criteria, 62 consented to participate. There was one twin pregnancy. Thirteen neonates were further excluded from analysis: eight for other possible reasons for neonatal thrombocytopenia (two with congenital cytomegalovirus infections, one with Jacobsen's syndrome, one with maternal immune thrombocytopenic purpura, one with neonatal hemochromatosis, one with Noonan's syndrome, one with Down's syndrome, one case of neonatal death 18 days after birth, where the autopsy showed underdeveloped bone marrow), and five cases where maternal sera were unavailable for antibody analysis. Thus, data from 50 cases over a period of 11 years were included for further analysis.

An unselected population of pregnant women originally included in a prospective study investigating maternal-fetal hemodynamics at the University Hospital of Northern Norway during the period 2006–2010 served as controls (Flo et al., 2010, 2014). Maternal blood samples were taken at 22–24 weeks of gestation. Additional maternal blood samples acquired within three days of delivery were available for seven controls. All samples were tested for the presence of maternal anti-HLA class I antibodies and categorized as either anti-HLA class I antibody-negative or -positive. Of 250 pregnancies in the control group, 72 (29%) tested positive for maternal anti-HLA class I antibodies. Platelet counts were obtained from 45 randomly selected neonates in the control group, none of which was thrombocytopenic.

All pregnancies were dated based on ultrasonography performed in the second trimester. Preeclampsia was diagnosed according to current ISSHP criteria (Tranquilli et al., 2014).

2.2. Definitions

Small for gestational age (SGA) was defined as birth weight less than the 10th percentile for gestational age based on singleton percentile curves (Skjaerven et al., 2000).

Infants born before 37 + 0 gestational weeks were defined as premature.

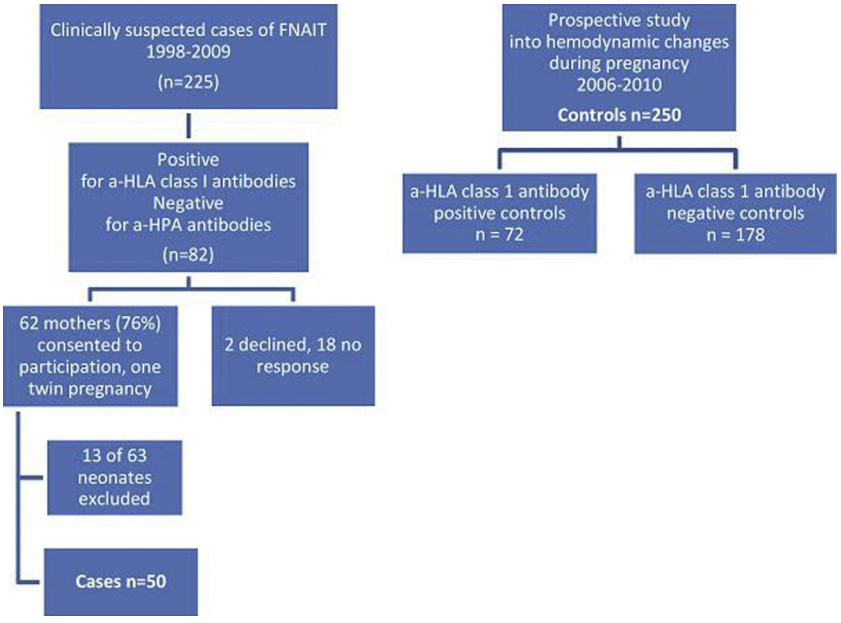

Fig.1. Cases consisted of pregnancies where the mother was anti-HLA class I antibody-positive and the neonate had suspected FNAIT, while controls consisted of normal pregnancies.

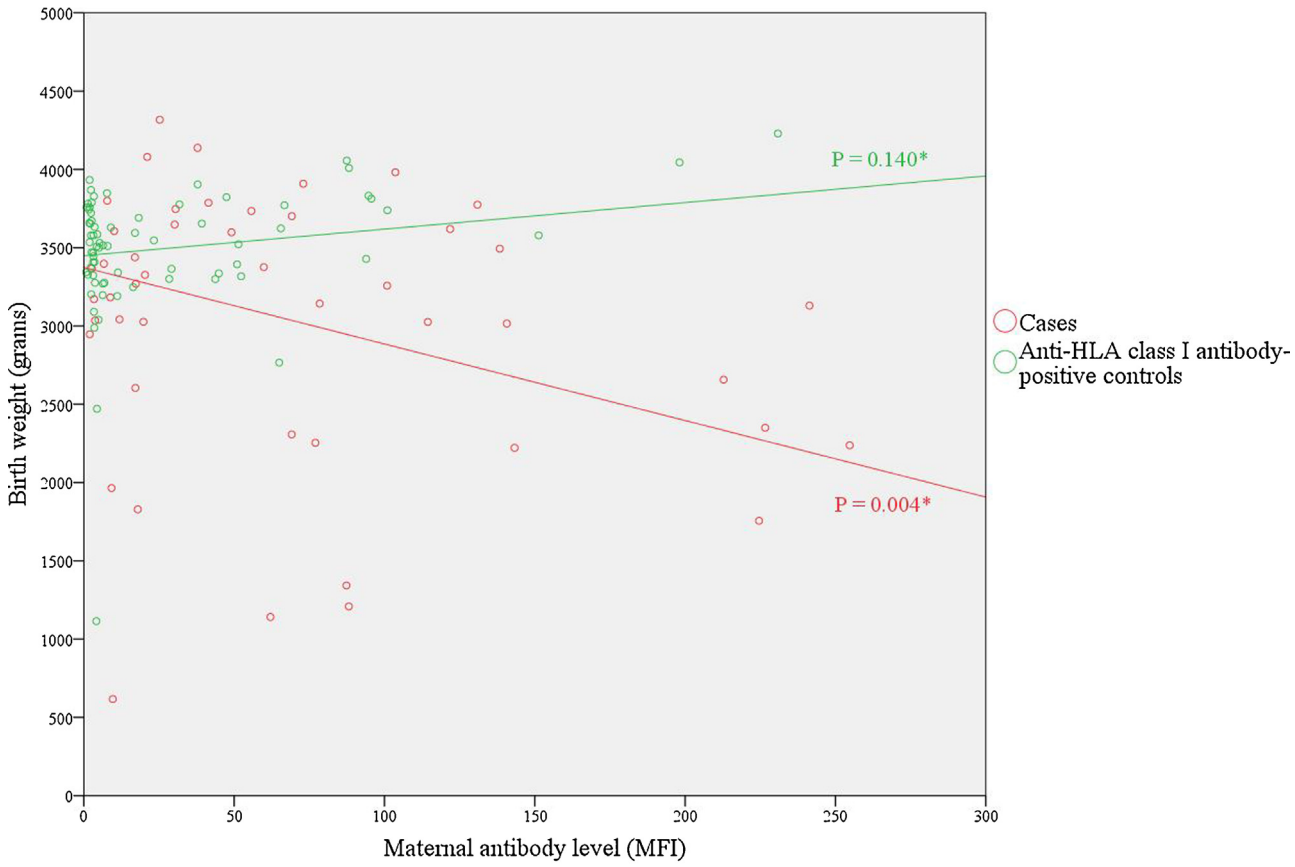

Fig. 2. Maternal antibody level versus predicted values for the birth weight in pregnancies with maternal anti-HLA class I antibodies and thrombocytopenic neonates (cases), and in normal pregnancies with maternal anti-HLA class I antibodies (anti-HLA class I antibody-positive controls). *Both regression lines adjusted for maternal age, parity, preeclampsia, gestational age at delivery, and sex of the fetus using multiple linear regression.

Table 1
Unadjusted maternal and neonatal characteristics for pregnancies with maternal anti-HLA class I antibodies and thrombocytopenic neonates (cases) versus normal pregnancies (controls).

	Cases	Controls	Anti-HLA class I antibody-positive controls	Anti-HLA class I antibody-negative controls
	(*n* = 50)	(*n* = 250)	(*n* = 72)	(*n* = 178)
Maternal age, mean (SD)	30.7 (5.1)[a]	29.1 (5.1)	29.8 (5.0)	28.9 (5.1)
Nulliparity, n (%)	21 (45)[a]	134 (54)	20 (28)	114 (64)
Smoking, n (%)	7 (20)[b]	16 (6)[a]	6 (8)[a]	10 (6)
Preeclampsia, n (%)	6 (13)[a]	15 (6)[b]	1 (1)	14 (8)[b]
Birth weight, grams (SD)	3005 (1039)[a]	3485 (638)[a]	3497 (563)	3480 (667)[a]
Gestational age at delivery, weeks^{days} (SD)	38^{3} (3^{1})[a]	39^{6} (2^{3})[a]	39^{6} (2^{2})	40^{0} (2^{3})[a]
SGA*, n (%)	23 (48)[a]	28 (11)[b]	7 (10)	21 (13)[b]
Placental weight, grams (SD)	607 (237)[b]	615 (144)[b]	621 (151)[a]	613 (141)[b]
PW/BW**, mean (SD)	0.208 (0.050)[b]	0.180 (0.039)[b]	0.179 (0.032)[a]	0.181 (0.041)[b]
MFI ratio, mean (SD)	68.8 (69.2)	9.1 (27.2)	28.4 (45.2)	1.1 (0.5)

([a]) 1–5 missing data (see Supplementary Table 2)

([b]) >5 missing data (see Supplementary Table 2)
(*) Small for gestational age
(**) Placental weight/birth weight ratio

Table 2
Comparison of birth weight, risk of SGA[a], placental weight, and PW/BW[b] between pregnancies with maternal anti-HLA class I antibodies and thrombocytopenic neonates (cases) versus normal pregnancies (controls).

Effect estimates of outcome variable							
Adjusting variable	Effect estimate (B) of birth weight in grams (95% CI)	*P*	Effect estimate (OR) of SGA (95% CI)	*P*	Effect estimate (B) of placental weight in grams (95% CI)	*P*	Effect estimate (B) of PW/BW (95% CI)	*P*
Study group (0 = controls, 1 = cases)	−167 (−323, −11)	0.036	6.72 (3.22, 14.03)	<0.001	−33.6 (−86.7, 19.5)	0.214	0.015 (0.003, 0.028)	0.012
Maternal age (years)	1.6 (−10.7, 13.9)	0.801	1.00 (0.93, 1.08)	0.991	1.1 (−2.6, 4.9)	0.557	0.000 (−0.001, 0.001)	0.803
Parity (0 = nulliparous, 1 = multiparous)	129 (5254)	0.042	0.62 (0.29, 1.32)	0.216	26.0 (−12.4, 64.5)	0.183	0.001 (−0.007, 0.010)	0.750
Preeclampsia (0 = no, 1=yes)	−102 (−328, 124)	0.373	5.01 (1.82, 13.82)	0.002	53.6 (−19.6, 126.7)	0.151	0.039 (0.022, 0.056)	<0.001
Gestational age at delivery (weeks)	213 (188, 237)	<0.001			31.6 (24.0, 39.2)	<0.001	−0.007 (−0.008, −0.005)	<0.001
Sex (0 = boy, 1 = girl)	−36 (−150, 77)	0.529	0.74 (0.38, 1.47)	0.396	5.2 (−30.0, 40.5)	0.770	−0.002 (−0.010, 0.006)	0.701

[a] Small for gestational age.
[b] Placental weight/birth weight ratio.

Thrombocytopenia was defined as a platelet count $<150 \times 10^9$/L. Severe thrombocytopenia was defined as a platelet count $<50 \times 10^9$/L.

A placental weight/birth weight ratio (PW/BW-ratio) was calculated and included in the analyses, as this ratio has been considered to be a better predictor of long-term fetal health than birth weight or placental weight alone (Hemachandra et al., 2006; Shehata et al., 2011).

2.3. Laboratory analysis

Screening for anti-HLA class I antibodies was done using an in-house monoclonal antibody immobilization of the platelet antigen (MAIPA) technique (Killie et al., 2010), or with FlowPRA 1 Screening Test (One Lambda, Canoga Park, CA, USA). In MAIPA, samples were tested against paternal platelets when available. If paternal platelets were not available, the study sample was tested against random donor platelets from at least four donors. All samples that tested negative for antibodies in MAIPA panels with random donor platelets were subsequently also tested using the FlowPRA 1 Screening Test (One Lambda) to uncover false negatives.

After the primary identification of anti-HLA class I antibody-positive cases, all samples were re-tested using the FlowPRA 1 Screening Test to obtain comparable values. A ratio of the median fluorescence intensity (MFI) for each sample to the MFI of the negative control for the assay was calculated and used when comparing anti-HLA class I antibody levels.

All laboratory work and analyses were performed by experienced bioengineers at the Norwegian National Unit for Platelet Immunology in Tromsø, Norway.

2.4. Statistics

All data were analyzed using SPSS software (version 21.0; SPSS, Chicago, IL, USA).

The normality of data distribution was tested using the Kolmogorov–Smirnov test. An independent samples *t*-test was used to compare means for continuous variables. Fisher's exact test was used to compare frequencies for categorical variables. A *P* value of <0.05 was considered significant, and 95% confidence intervals (CI) were reported where appropriate.

Regression models were applied to further evaluate differences in birth weight, frequency of SGA, placental weight and the PW/BW ratio between study groups. In all regression models we adjusted for maternal age, parity (nulli- or multiparity), preeclampsia (yes/no), sex of the fetus, and gestational age at the time of delivery. When looking at associations among maternal antibody level and birth weight, SGA, placental weight, or PW/BW within each study group, maternal antibody level was included as an independent variable. We did not adjust for gestational age at delivery when SGA was defined as the dependent variable.

Missing data were treated by pairwise deletion when comparing all unadjusted data and by list-wise deletion when performing regression analyses.

Data on smoking habits were available for 70% of cases. All regression analyses were performed both with and without adjusting for smoking habits. Data presented in the results did not take into account smoking habits unless otherwise stated.

2.5. Ethics

The study was approved by the Regional Committee for Medical Research Ethics, North Norway (Ref. no. REKNORD 2013/1863: date of approval 15.05.2014). Informed written consent was obtained from all women included in this project.

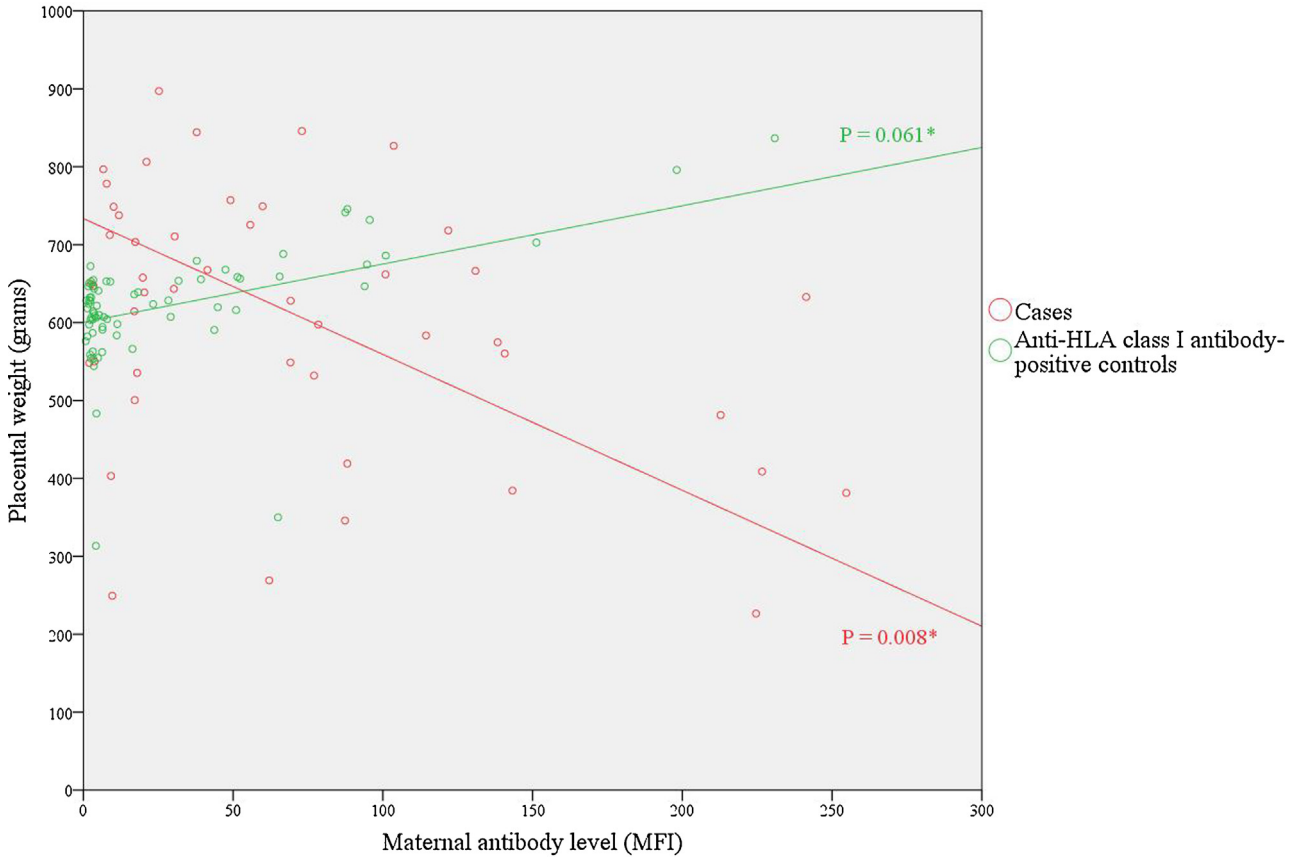

Fig. 3. Maternal antibody level versus predicted values for the placental weight in pregnancies with maternal anti-HLA class I antibodies and thrombocytopenic neonates (cases), and in normal pregnancies with maternal anti-HLA class I antibodies (anti-HLA class I antibody-positive controls). *Both regression lines adjusted for maternal age, parity, preeclampsia, gestational age at delivery, and sex of the fetus using multiple linear regression.

3. Results

Children in the case group had a significantly lower birth weight (3005 g versus 3485 g, $P = 0.003$) and a higher frequency of being SGA (48% versus 12%, $P < 0.001$) compared with controls. Almost 20% of infants (9/48) in the case group were born premature, compared with 5% among controls ($P = 0.004$). There was no significant difference in placental weight between cases and controls. Five of the neonates (10%) in the case group had ICH. Clinical characteristics did not differ significantly between the two control groups, except for the frequency of nulliparous mothers. Maternal and neonatal characteristics are presented in Table 1.

Mean adjusted birth weight among cases was significantly lower compared with controls (adjusted weight difference 167 g, $P = 0.036$, Table 2). The risk of being SGA was significantly higher among cases than among controls (OR = 6.72, $P < 0.001$, Table 2). In contrast, there was no significant difference in adjusted birth weight or frequency of SGA between anti-HLA class I antibody-positive and -negative controls (data not shown). There were no significant adjusted differences between boys and girls for birth weight, risk of SGA or placental weight in any of our study groups (data not shown).

Mean maternal anti-HLA class I antibody level was significantly higher among cases compared with anti-HLA class I antibody-positive controls (unadjusted, $P = 0.001$, Table 1). Further, the mean maternal anti-HLA class I antibody level was significantly higher in cases where the infant was SGA at delivery compared with cases where the infant was not SGA (unadjusted, $P = 0.037$, data not shown). An increasing level of anti-HLA class I antibodies was significantly associated with decreasing birth weight among cases ($\beta = -4.9$, $P = 0.004$, Fig. 2). Although non-significant, the trend among anti-HLA class I antibody-positive controls was opposite, with the level of maternal anti-HLA class I antibodies increasing together with increasing birth weight ($\beta = 1.7$, $P = 0.140$, Fig. 2). There was a significant association between maternal anti-HLA class I antibody level and frequency of SGA among cases when smoking was included as an independent variable (OR = 1.026, $P = 0.021$, data not shown). This association was not significant when smoking was not included in the regression model (OR = 1.011, $P = 0.058$, data not shown).

An increasing level of anti-HLA class I antibodies was further significantly associated with decreasing placental weight among cases ($\beta = -1.7$, $P = 0.008$). This association had an opposite trend among anti-HLA class I antibody-positive controls, although it was non-significant ($\beta = 0.748$, $P = 0.061$) (Fig. 3). The cases had a significantly higher PW/BW ratio than controls (mean adjusted difference 0.015, $P = 0.012$). There was no significant association between maternal anti-HLA class I antibody level and PW/BW ratio for any of the study groups (data not shown).

The frequency of preeclampsia among cases was 13%. Five out of six infants whose mother had preeclampsia were SGA at birth. Because of the known association between preeclampsia and fetal growth restriction, we repeated the analyses after excluding all preeclamptic pregnancies; lower birth weight, and the risk of having an SGA infant were still significantly and similarly associated with maternal anti-HLA class I antibodies among cases (data not shown).

To evaluate the use of MFI from the FlowPRA I Screening Test as a quantitative measure, we compared these results with those of the MAIPA assays. We found a strong degree of correlation between

the optical density value obtained from MAIPA and the MFI from FlowPRA (Spearman's correlation coefficient = 0.680, $P < 0.001$).

As the sampling time varied between the case and the control group, we evaluated whether sampling time could have influenced the observed difference in anti-HLA class I antibody levels. Samples taken both antenatally and postpartum were available from seven participants in the anti-HLA class I antibody-positive control group. Samples taken at weeks 22–24 had an MFI that was on average 66% *higher* than the MFI of the postpartum samples (mean 1.66, 95% CI 0.94–2.38). Although not statistically significant, the trend indicates that the anti-HLA class 1 antibody level tends to fall after delivery. If this trend is representative, the difference in antibody level between cases and controls would have been even larger if samples had been taken at the same time in both study groups.

Neonatal platelet counts were available for 46 of the 50 neonates in the case group. The mean platelet count in this group was 29×10^9/L (SD 19×10^9/L). The mean platelet count in the umbilical cord blood of 45 randomly selected controls (antibody positive and antibody negative) was 290×10^9/L ($n = 45$, SD 62×10^9/L). None of the controls had thrombocytopenia.

4. Discussion

4.1. Main findings

Thrombocytopenic neonates born to mothers with anti-HLA class I antibodies had a significantly lower birth weight compared with controls. An increasing level of maternal anti-HLA class I antibodies was linearly and inversely associated with birth weight and placenta weight among the thrombocytopenic neonates. There was no difference in birth weight between the antibody-positive and antibody-negative controls.

4.2. Strengths and limitations

Considering the rarity of the condition we describe, the size of our study population is large and with few missing data (Table S1). The cases were selected from nation-wide referrals of pregnancies where FNAIT was suspected. As FNAIT investigations are undertaken only at the Norwegian National Unit for Platelet Immunology, the case group is considered representative of Norwegian FNAIT pregnancies in general. Most of the neonates in the case group had severe thrombocytopenia. A selection bias toward more severe cases is possible owing to the retrospective study design. To assess the external validity of the control group, the clinical variables were compared with equivalent data from the Medical Birth Registry of Norway (MBRN) for 2010 (Norwegian, 2012). Except for a lower frequency of smokers (6.4% versus 18.5% in the MBRN), all maternal and neonatal characteristics were similar to data from the MBRN (Table S2). The controls included in this study may therefore be considered representative of a normal background population of Norwegian pregnancies. The participants in both groups were originally identified prospectively and during overlapping time periods. We therefore consider the two groups comparable. The majority of our study participants were White European. Our findings may therefore not be representative of other ethnicities.

To remove possible confounders, we excluded all cases where other possible causes of thrombocytopenia could be found in the patient's medical records, but undisclosed causes could still be present.

The frequency of ICH among cases was 10%, which is similar to what has been previously reported for children with severe FNAIT (Mueller-Eckhardt et al., 1989; Kamphuis et al., 2010). The frequency of maternal anti-HLA class I antibodies among controls was 29%, which is also in accordance with previous reports (Morin-Papunen et al., 1984; Regan et al., 1991; King et al., 1996; Masson et al., 2013). Considering that neonatal infections are a common cause of neonatal thrombocytopenia, the number of neonates in the case group excluded because of neonatal infections was low. However, newborns diagnosed with infection typically would not be referred for FNAIT investigation, thus explaining the low number of infections in our study population.

We used fluorescence intensities obtained from the FlowPRA I Screening Test as a measure of anti-HLA class I antibody level. The panel of antigens present in the FlowPRA I Screening Test contains all of the most common and several rare HLA class I antigens described in our population. However, some of the antigens are represented at different frequencies, meaning that certain antibody specificities have more binding sites than others. The FlowPRA I Screening Test is not validated as a quantitative test by the manufacturer, although MFI is commonly considered a semi- quantitative measure of antibody level (Akalin et al., 2008; Lefaucheur et al., 2010). The antibody level results in this study must be interpreted with caution. The essence of our data is primarily that antibody level seems to matter, but future assays designed to measure anti-HLA class I antibody level are warranted.

4.3. Interpretation

The association between maternal anti-HLA class I antibodies and birth weight in relation to neonatal thrombocytopenia has not been described before. However, a similar association between maternal antibodies against HPA-1a and reduced birth weight in boys has been reported (Tiller et al., 2012), and neonatal thrombocytopenia in general is commonly detected in growth-retarded newborns (Beiner et al., 2003; Engineer and Kumar, 2010). The lack of significant associations between maternal anti-HLA class I antibodies and birth weight or placental weight among controls could indicate that anti-HLA class I antibodies alone do not influence fetoplacental growth. It is possible that the lower birth weight was mediated through fetal thrombocytopenia, rather than through the anti-HLA class 1 antibodies. The observation that antibody levels were much higher among cases than among controls makes it difficult to draw any conclusions on this matter. Prospective studies are needed to clarify whether there could be a threshold level of anti-HLA class I antibodies above which fetoplacental growth may be affected.

Alternatively, both the maternal anti-HLA class 1 antibodies and the neonatal thrombocytopenia may have been epiphenomena, with an undisclosed third factor actually causing the lower birth weight. A previous study reported that the breadth and strength of anti-HLA antibodies increased with inflammation in transplanted patients (Locke et al., 2009), and it was also recently shown that antibody-mediated platelet destruction by human phagocytes is enhanced by the inflammatory marker C-reactive protein (CRP) (Kapur et al., 2015). Both the observed thrombocytopenia and the high anti-HLA class I antibody levels among cases may therefore be secondary to an increased state of inflammation caused by other factors. Even so, their presence may still have affected fetoplacental growth.

Most reports on neonatal thrombocytopenia and low birth weight are based on the assumption that thrombocytopenia is secondary to reduced fetal growth and/or placental failure. It is noteworthy that the possibility that the cause–effect may be the other way around has not been considered. Therefore, it is not possible to assess or rule out the role of maternal alloantibodies in association with neonatal thrombocytopenia in fetal growth restriction based on previous studies. The observed association in this study between increasing anti-HLA class I antibody level and decreasing birth weight and placental weight is interesting and supports the hypothesis that maternal anti-HLA class I antibod-

ies might affect fetal growth, either directly or indirectly via fetal thrombocytopenia. Future research including placental studies is warranted to test these hypotheses.

Anti-HLA class I antibodies are directed against antigens with a high degree of polymorphism. The distribution of antibody specificities in this study may therefore have differed between cases and controls, thus contributing to the differences observed. Additional analysis of antibody specificities would clarify this.

The frequency of preeclampsia among cases was unexpectedly high at 13%. After removal of all preeclamptic pregnancies from our analyses the described results did not change, supporting the theory that other factors in the case group (i.e., anti-HLA class 1 antibodies or fetal thrombocytopenia) were mainly responsible for the lower birth weight. The high frequency of preeclampsia among HLA class I-immunized pregnancies in relation to neonatal thrombocytopenia is still interesting. An increased frequency of preeclampsia among mothers having preterm infants with thrombocytopenia was previously reported (Beiner et al., 2003), and a possible connection between the maternal production of antibodies during pregnancy and the development of preeclampsia has been suggested (Buurma et al., 2012). An exacerbated maternal inflammatory response is found in preeclamptic pregnancies (Redman and Sargent, 2004), and inflammation has further been shown to increase the breadth and strength of anti-HLA class I antibodies (Locke et al., 2009). There was no association between anti-HLA class I antibodies and preeclampsia among controls, but the different selection strategies of the study groups may have influenced this. The possible link between maternal anti-HLA class I antibodies and preeclampsia should be further studied.

As HLA antibodies are common and neonatal thrombocytopenia occurs relatively frequently, it is difficult to draw a definite conclusion based on this observational study. However, the hypotheses generated from our study deserve to be further evaluated.

5. Conclusions

Maternal anti-HLA class I antibodies are associated with reduced birth weight in neonates with suspected FNAIT. Further studies are needed to disclose whether maternal anti-HLA class I antibodies occurring during pregnancy can affect fetal growth, or if their presence is simply an epiphenomenon.

Conflict of interest

We have no conflicts of interest.

Acknowledgements

The authors wish to thank Kristine Tollefsen at University Hospital North Norway and Eirin Listau Bertelsen at UiT, the Arctic University of Norway, for help with laboratory analysis. We are also grateful to Åse Vårtun at University Hospital North Norway for assistance with providing plasma samples from the control group. Funding was obtained from the Northern Norway Regional Health Authority.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jri.2015.10.003.

References

Akalin, E., et al., 2008. Addition of plasmapheresis decreases the incidence of acute antibody-mediated rejection in sensitized patients with strong donor-specific antibodies. Clin. J. Am. Soc. Nephrol.: CJASN 3, 1160–1167.

Arck, P.C., Hecher, K., 2013. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. Nat. Med. 19, 548–556.

Beiner, M.E., et al., 2003. Risk factors for neonatal thrombocytopenia in preterm infants. Am. J. Perinatol. 20, 49–54.

Buurma, A., et al., 2012. Preeclampsia is characterized by placental complement dysregulation. Hypertension 60, 1332–1337.

Davoren, A., et al., 2004. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion 44, 1220–1225.

Dreyfus, M., et al., 1997. Frequency of immune thrombocytopenia in newborns: a prospective study. Immune thrombocytopenia working group. Blood 89, 4402–4406.

Engineer, N., Kumar, S., 2010. Perinatal variables and neonatal outcomes in severely growth restricted preterm fetuses. Acta Obstet. Gynecol. Scand. 89, 1174–1181.

Flo, K., et al., 2010. A longitudinal study of the relationship between maternal cardiac output measured by impedance cardiography and uterine artery blood flow in the second half of pregnancy. BJOG: Int. J. Obstet. Gynaecol. 117, 837–844.

Flo, K., et al., 2014. Blood flow to the scarred gravid uterus at 22–24 weeks of gestation. BJOG: Int. J. Obstet. Gynaecol. 121, 210–215.

Gramatges, M.M., et al., 2009. Neonatal alloimmune thrombocytopenia and neutropenia associated with maternal human leukocyte antigen antibodies. Pediatr. Blood Cancer 53, 97–99.

Hemachandra, A.H., et al., 2006. The association between intrauterine growth restriction in the full-term infant and high blood pressure at age 7 years: results from the collaborative perinatal project. Int. J. Epidemiol. 35, 871–877.

Kamphuis, M.M., et al., 2010. Screening in pregnancy for fetal or neonatal alloimmune thrombocytopenia: systematic review. BJOG: Int. J. Obstet. Gynaecol. 117, 1335–1343.

Kapur, R., et al., 2015. C-reactive protein enhances IgG-mediated phagocyte responses and thrombocytopenia. Blood 125, 1793–1802.

Killie, M.K., et al., 2010. Quantitative MAIPA: comparison of different MAIPA protocols. Transfus. Apheresis Sci. 43, 149–154.

King, K.E., et al., 1996. The role of HLA antibodies in neonatal thrombocytopenia: a prospective study. Tissue Antigens 47, 206–211.

Kjeldsen-Kragh, J., et al., 2007. A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. Blood 110, 833–839.

Lashley, E.E., et al., 2013. Beneficial or harmful effect of antipaternal human leukocyte antibodies on pregnancy outcome? A systematic review and meta-analysis. Am. J. Reprod. Immunol. 70, 87–103.

Lee, P.C., et al., 2002. All chronic rejection failures of kidney transplants were preceded by the development of hla antibodies. Transplantation 74, 1192–1194.

Lefaucheur, C., et al., 2010. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J. Am. Soc. Nephrol.: JASN 21, 1398–1406.

Locke, J.E., et al., 2009. Proinflammatory events are associated with significant increases in breadth and strength of HLA-specific antibody. American J. Transplant. 9, 2136–2139.

Masson, E., et al., 2013. Incidence and risk factors of anti-HLA immunization after pregnancy. Hum. Immunol. 74, 946–951.

Moncharmont, P., et al., 2004. HLA antibodies and neonatal alloimmune thrombocytopenia. Acta Haematol. 111, 215–220.

Morin-Papunen, L., et al., 1984. Maternal HLA immunization during pregnancy: presence of anti HLA antibodies in half of multigravidous women. Med. Biol. 62, 323–325.

Mueller-Eckhardt, C., et al., 1989. 348 Cases of suspected neonatal alloimmune thrombocytopenia. Lancet 1, 363–366.

Nielsen, H.S., et al., 2010. The presence of HLA-antibodies in recurrent miscarriage patients is associated with a reduced chance of a live birth. J. Reprod. Immunol. 87, 67–73.

Norwegian Institute of Public Health, 2012. Fødsler i norge 2010.

Novotny, V.M., 1999. Prevention and management of platelet transfusion refractoriness. Vox Sang. 76, 1–13.

Redman, C.W., Sargent, I.L., 2004. Preeclampsia and the systemic inflammatory response. Semin. Nephrol. 24, 565–570.

Regan, L., et al., 1991. A prospective study of the incidence: time of appearance and significance of anti-paternal lymphocytotoxic antibodies in human pregnancy. Hum. Reprod. 6, 294–298.

Saito, S., et al., 2003. Serologic analysis of three cases of neonatal alloimmune thrombocytopenia associated with HLA antibodies. Transfusion 43, 908–917.

Sargent, I.L., et al., 1988. Maternal immune responses to the fetus in early pregnancy and recurrent miscarriage. Lancet 2, 1099–1104.

Shehata, F., et al., 2011. Placenta/birthweight ratio and perinatal outcome: a retrospective cohort analysis. BJOG: Int. J. Obstet. Gynaecol. 118, 741–747.

Skjaerven, R., et al., 2000. Birthweight by gestational age in norway. Acta Obstet. Gynecol. Scand. 79, 440–449.

Skogen, B., et al., 2010. Reconsidering fetal and neonatal alloimmune thrombocytopenia with a focus on screening and prevention. Expert Rev. Hematol. 3, 559–566.

Starcevic, M., et al., 2010. Neonatal alloimmune thrombocytopenia caused by anti-HLA-A24 alloantibodies. Acta Paediatr. 99, 630–632.

Steinborn, A., et al., 2004. Anti-fetal immune response mechanisms may be involved in the pathogenesis of placental abruption. Clin. Immunol. 110, 45–54.

Thude, H., et al., 2006. Neonatal alloimmune thrombocytopenia caused by human leucocyte antigen-B27 antibody. Transfus. Med. 16, 143–149.
Tiller, H., et al., 2012. Platelet antibodies and fetal growth: maternal antibodies against fetal platelet antigen 1a are strongly associated with reduced birthweight in boys. Acta Obstet. Gynecol. Scand. 91, 79–86.
Tranquilli, A.L., et al., 2014. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. Pregnancy Hypertens. 4, 97–104.
Zhang, Q., et al., 2005. Development of posttransplant antidonor HLA antibodies is associated with acute humoral rejection and early graft dysfunction. Transplantation 79, 591–598.