KIR and HLA-C: Immunogenetic regulation of human birth weight

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ABSTRACT

Pregnancies resulting in very small or very large babies are at higher risk of obstetric complications with increased morbidity for both mother and baby. Using data from the Medical Birth Registry of Norway we have shown how human birth weight is still subject to stabilizing selection. Particular combinations of maternal/fetal immune genes have been implicated in pregnancies resulting in a low birth weight baby (<5th birth weight centile). More specifically, an inhibitory maternal *KIRAA* genotype with a paternally derived fetal *HLA-C2* ligand. At the other end of the birth weight spectrum the presence of an activating maternal *KIR2DS1* gene is associated with increased birth weight in linear or logistic regression analyses of all pregnancies >5th centile (p=0.005, OR=2.65). Thus, inhibitory maternal *KIR* combined with fetal *HLA-C2* is more frequently associated with low birth weight, whereas activating maternal *KIR* with fetal *HLA-C2* ligand is associated with increasing birth weight. Our findings using the MoBa cohort have replicated the association of *KIR* and *HLA-C* seen in poor placentation, and confirm the importance of maternal/fetal immune gene interactions in determining the outcome of pregnancy.

Our work aims to understand how the immune system contributes to successful pregnancy. The placenta forms the interface between the mother and baby in the uterus. Fetal extravillous trophoblast cells (EVT) invade into the maternal decidua where they remodel uterine spiral artery walls converting them into high conductance vessels, thus securing a good blood supply to the feto-placental unit [1]. Insufficient transformation of the maternal spiral arteries results in poor placental development which consequently gives rise to disorders of pregnancy such as pre-eclampsia, fetal growth restriction (FGR), still birth and recurrent miscarriage [2-4]. Excessive invasion by EVT can also be detrimental in cases such as placenta accreta. High birth weight is potentially dangerous to both mother and baby through prolonged obstructed labour [5,6] with risk of asphyxia and injury in the baby [7-9], and lacerations and post-partum haemorrhage in the mother [5,9]. It is therefore crucially important that this process is carefully regulated maintaining a balance between over and under invasion. Using data from 795,068 first pregnancies resulting in a live birth from the Medical Birth Registry of Norway, we plotted the distribution of birth weight against frequency of transfer to special care baby unit [10]. This illustrated clearly the risk of morbidity in neonates at either end of the birth weight spectrum, even in a modern medicalised setting (Fig. 1).

Large numbers of a specialised type of lymphocyte known as uterine NK cells are found in the decidua during placentation [11]. Uterine NK cells express Killer cell Ig-like Receptors (KIR) and fetal EVT express their cognate ligand HLA-C, the only classical HLA class I molecule found on trophoblast [12]. We have proposed that this maternal KIR/fetal HLA-C interaction functions to mediate uterine NK cell control of trophoblast invasion [11,13-15].

This receptor ligand interaction is unusual in that both *KIR* and *HLA* are highly polymorphic gene systems. They also segregate independently and are encoded on separate chromosomes. *KIR* genotypes vary with content, copy number and at allelic variation at individual *KIR* loci. Around 500 different genotypes have already been described to date [16]. To simplify this complexity, *KIR* haplotypes are classified as either *A* or *B* based on gene content. The *KIR A* haplotype is highly stable, varying little at the gene content level and carrying fewer genes. Notably it encodes *KIR2DL1* and *KIR2DL3*, both inhibitory receptors for HLA-C. The *KIR B* haplotype is much more variable with the potential to encode inhibitory *KIR2DL1* and *KIR2DL2*, and also activating *KIR2DS1*, all of which bind HLA-C [11].

HLA-C alleles can be subdivided into two groups C1 and C2 based on a dimorphism at position 80 of the

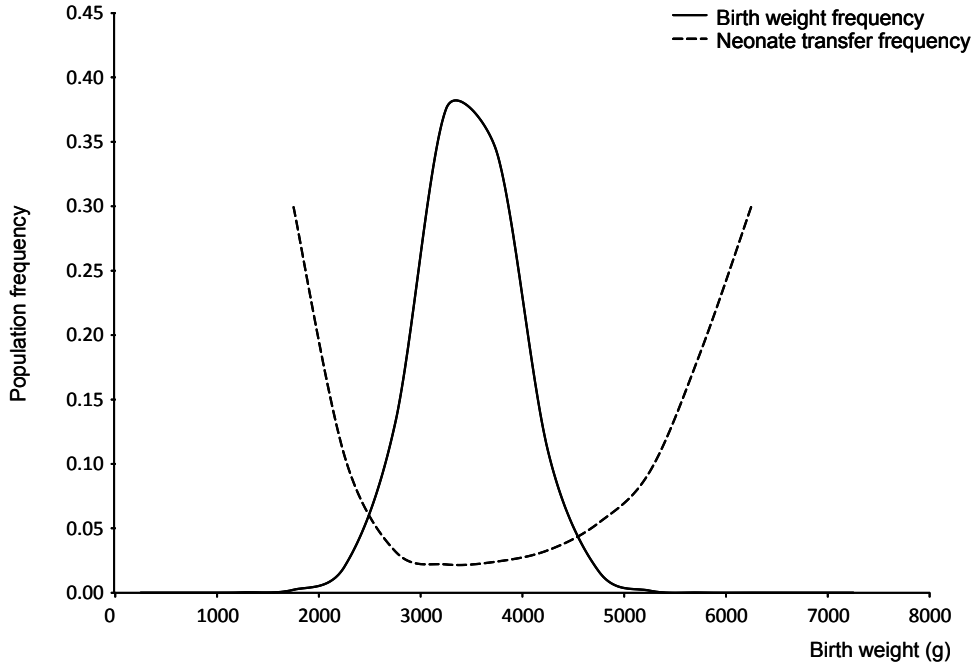

Figure 1. Distribution of birth weights in the Norwegian population with percentage of babies transferred to the special care baby unit for the years 1967-2010 (n=795,068). (Originally published in *Journal of Immunology*: Hiby S, Apps R, Chazara O, et al. Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. *J. Immunol* 2014; **192**: 5069-5073. Copyright © [2014] The American Association of Immunologists, Inc.)

α1 domain [17]. In this way KIR distinguish between C1 and C2 as mutually exclusive epitopes (Table 1).

To determine if maternal *KIR* and fetal *HLA-C* variation is important in pregnancy we have looked for consistent genetic associations of particular maternal *KIR* and fetal *HLA-C* combinations in a range of obstetric disorders (pre-eclampsia, FGR, and recurrent miscarriage) all of which result from defective placentation. All these conditions share a genetic association characterised by mothers with two *KIR A* haplotypes (*AA* genotype), combined with an *HLA-C2* group allele in the fetus, particularly when the fetal *C2* is paternally derived. Conversely, women who carry a *KIR B* haplotype encoding *KIR2DS1*, the activating receptor for C2, are significantly protected from these disorders when a fetal *C2* is present [11,18,19].

Because we were intrigued by the high fetal and maternal mortality and morbidity seen in pregnancies with high birth weights [5-9] we went on to question what maternal *KIR* and fetal *HLA-C* combinations were associated with these pregnancies.

Mother and baby DNA pairs were genotyped for *KIR* and *HLA-C* from two separate cohorts. From our United Kingdom cohort we analysed 747 pre-eclamptic pregnancies, 118 pregnancies with FGR ($\leq 5^{th}$ birth weight centile) and 404 normal pregnancies [10]. Selected from the MoBa cohort [20] were 995 normal (including 66 $>90^{th}$ birth weight centile), and 141 pre-eclamptic first pregnancies.

Replicating our previous findings from the UK, increased maternal *KIR AA* and low *KIR2DS1* frequencies associated with pregnancies with low birth weight and/or pre-eclampsia in the MoBa cohort. Conversely, high birth weight pregnancies ($\geq 90^{th}$ centile) had low *KIR AA* and high *KIR2DS1* frequencies [10].

The effect of *KIR2DS1* on birth weight was tested in both categorical and continuous analysis. Using birth weight in grams as a continuous variable, the presence of a maternal *KIR2DS1* conferred an average birth weight increase of 78g (p=0.005) in a linear regression model. The frequency of maternal *KIR2DS1* was significantly higher in pregnancies with high compared with median birth weight in categorical analysis (Table 2, [10]).

The effect of KIR *AA* genotypes on pre-eclampsia and FGR was previously observed particularly in pregnancies with a fetus carrying a paternally derived *C2*. This was shown by both categorical and continuous analysis across the birth weight spectrum. The effect of maternal *KIR2DS1* on birth weight is thus dependent on the presence of fetal *C2*, particularly paternally derived fetal *C2*.

In other words the presence of fetal *C2* amplifies the effect of maternal *KIR2DS1*. When the fetus has more *C2* groups than the mother (*C1/C2* fetus with *C1/C1* mother, and *C2/*C2 fetus with *C1/C2* mother), an average increase of 245g (p=0.002) was seen. Furthermore, presence or absence of *KIR2DS1* as a categorical variable when combined with more *C2* in the fetus showed that maternal *KIR2DS1* has a significant effect when the fetus is carrying more *C2* than the mother, (OR 2.93, 95% CI 1.66-5.18, p=0.0002). In pregnancies where the fetus was *C1/C2* we could determine parent of origin of the *C2* group. In both categorical and continuous analyses, the presence of maternal *KIR2DS1* only has an effect on birth weight

Table 1. KIR known to bind HLA-C and their HLA-C ligands.

KIR	HLA-C Ligand	*KIR* Haplotype location	Activating/Inhibitory
2DL1	C2	*A* and some *B*	Inhibitory
2DL2	C1, some C2	*B*	Inhibitory
2DL3	C1	*A*	Inhibitory
2DS1	C2	*B*	Activating
2DS2	Possibly C1	*B*	Activating
2DS4	Some C1 and some C2	*A* (often deleted)	Activating

Table 2. Presence of maternal *KIR2DS1* associates with increased birth weight in both categorical and continuous analysis n=1316. This is enhanced when the fetus has more *HLA-C2* epitopes than the mother n=304 and specifically paternally derived *C2* n=204. Data summarised from tables I, II, and IV-VII in Hiby S, Apps R, Chazara O, et al. Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. *J. Immunol* 2014; **192**: 5069-5073.

	Continuous analysis		Categorical analysis		
	Weight	p-value	OR	95% CI	p-value
Presence of maternal *KIR2DS1*	78 g ↑	0.005	1.38	1.07-1.79	0.01
Maternal *KIR2DS1* with more *C2* in fetus than mother	245 g ↑	0.0002	2.93	1.66-5.18	0.0002
Maternal *KIR2DS1* with paternally derived fetal *C2*	196 g ↑	0.016	2.65	1.33-5.26	0.005

when the fetal *C2* is paternally derived (paternal *C2* p=0.016, maternal *C2* p=0.75) (Table 2).

Our findings using MoBa subjects have replicated the association of *KIR* and *HLA-C* with poor placentation (pre-eclampsia, and low birth weight) and confirm the importance of maternal KIR/fetal HLA-C interactions in determining the outcome of pregnancy. Of importance is that we now also show an effect in high birth weight pregnancies, implicating a role in the regulation of placentation in normal or excessive invasion. This effect has a clear direct impact on birth weight as a continuous variable, with an effect comparable or even greater than smoking during pregnancy, high altitude and sex of the baby [21-24].

KIR2DS1 is expressed by uterine NK cells and is functional although ascertaining exactly how NK cells operate to subtly define the extent of arterial transformation by trophoblast is an exciting challenge for the future. Nonetheless our findings indicate that a balance of KIR inhibitory and activating stimuli is necessary for optimal trophoblast invasion. NK derived cytokines such as GM-CSF, released in response to activation of KIR2DS1 by binding C2, are one possible mechanism [13].

Not all women with *KIR AA* genotypes and fetal *C2* have a pregnancy disorder. We are now selecting patients from the MoBa cohort to study women who have recurrent pre-eclampsia as well as those who have a normal pregnancy followed by a pre-eclamptic pregnancy and vice versa. We aim to focus on KIR2DL1, the inhibitory KIR for HLA-C2 because there are 4 different *KIR2DL1* alleles in the Norwegian population. Our prediction is that particular *KIR2DL1* alleles will confer most risk.

Perhaps in the long term *KIR/HLA-C* genotyping might be a genetic predictor of birth weight to identify those at risk of FGR, pre-eclampsia or macrosomia.

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Figure 1 reproduced with kind permission of the Journal of Immunology. This work was supported by Wellcome Trust Grants 090108/Z/09/Z and 085992/Z/08/Z, British Heart Foundation Grant PG/09/077/27964, and the Centre for Trophoblast Research. This work was also supported by Frederick National Laboratory for Cancer Research Contract HHSN261200800001E and by the Intramural Research Program of National Institutes of Health, Frederick National Laboratory, Center for Cancer Research. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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