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The role of the maternal immune system in the regulation of human birth weight

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Summary

Human birth weight is subject to stabilizing selection. Large babies are at risk of obstetric complications such as obstructed labour, which endangers both mother and child. Small babies are also at risk with reduced survival. Fetal growth requires remodeling of maternal spiral arteries to provide an adequate maternal blood supply to the placenta. This arterial transformation is achieved by placental trophoblast cells, which invade into the uterine wall. Under invasion is associated with fetal growth restriction; but if invasion is excessive large babies can result. A growing body of evidence suggests that this process is controlled by interactions between KIR receptors expressed on maternal uterine NK cells (uNK) and their corresponding HLA-C ligands on invading trophoblast. Mothers with the *KIR AA* genotype and a fetus with a paternal *HLA-C2* allele tend to have small babies, because this combination inhibits cytokine secretion by uNK. Mothers with the activating *KIR2DS1* gene and an *HLA-C2* fetus are more likely to have large babies. When KIR2DS1 binds to HLA-C2 this increases secretion of cytokines that enhance trophoblast invasion. We conclude that specific combinations of the highly polymorphic gene systems, *KIR* and *HLA-C*, contribute to successful reproduction by maintaining birth weight between two extremes.

Index words/phrases:
Birth weight, Natural Killer (NK) cells, immunology, pre-eclampsia, fetal growth restriction, placental development

Introduction

The role of the immune system in the development of both the brain and the placenta during the evolution of modern humans may not be immediately obvious. This paper reviews the part played by the immune system at the moment in human life history when brain size and pelvic anatomy interact in a way that is critical to success and failure of pregnancy. Human brains are now so large that it is an extremely tight fit for them to squeeze through the pelvis. Indeed, babies often do get stuck during the passage through the birth canal resulting in obstructed labour. It is not just pregnancies with very large babies where mothers and their babies are at risk of morbidity and mortality. At the opposite end of the spectrum when babies are too small this can have equally damaging consequences. Karn and Penrose first noted this selective pressure at the extremes of the birth weight spectrum in the 1930s but it was Bodmer who pointed out that human birth weight is a prime example of balancing or stabilizing selection (1,2). Although death of babies is now less frequent due to advances in neonatal medicine, there is still considerable morbidity. Our recent study in Norway shows babies were most frequently admitted to the neonatal ward when they were born at the extremes of the birth weight spectrum (3). This suggests there is still strong selective pressure to maintain human birth weight between these two extremes. This situation, encapsulated in the notion of the Obstetric Dilemma, is a particular problem for humans.

Placentation and fetal growth

Growth of the fetus and size at birth must depend on the delivery of sufficient nutrients and oxygen to the placenta. The supply line in the uterus is via the maternal arteries supplying blood to the placenta – so-called spiral arteries. In humans these are structurally transformed during early pregnancy to allow blood flow to increase about 100-fold (4). Arterial transformation depends on an unusual process during placentation, whereby fetal trophoblast cells from the placenta infiltrate into the uterine wall, home to the arteries and destroy the smooth muscle media. As a result of this trophoblast modification, the arteries become conduits capable of high

conductance at low pressure with a reduction in velocity of the blood entering the placenta. Terminal dilatation of the arteries results in a further reduction in the flow rate into the intervillous space. This permits adequate time for gas exchange, especially towards the end of the pregnancy when fetal demands are highest. When there is failure of arterial conversion by trophoblast, then arterial blood will jet into the intervillous space from the non-transformed arteries causing damage to the villous tree (Figure 1). Transport of oxygen and nutrients to the fetus is reduced and this manifests as fetal growth restriction (FGR). In more severe cases the mother may develop pre-eclampsia, a systemic syndrome that occurs when the placenta becomes stressed as a result of the reduced blood flow (5). Soluble factors released from the stressed placenta can trigger an inflammatory condition with diverse clinical manifestations. These include oedema, proteinuria and high blood pressure and can progress to eclampsia and maternal death. This is one of the principal reasons for clinical problems in both mothers and babies in pregnancies with very low birth weights. There are other clinical conditions besides pre-eclampsia and FGR resulting from defective placentation. Known collectively as the Great Obstetric Syndromes (GOS), they include unexplained stillbirth, placental abruption and preterm labour (6).

The Obstetric Dilemma

Very large babies (>~4kg) represent the other extreme of the birth weight spectrum and these pregnancies are also at risk of clinical problems due to the difficulty of the passage of their head through the pelvis. Fetal obstruction results in prolonged labour, fetal death from asphyxia, soft tissue damage to pelvic organs and post-partum haemorrhage (7). Estimation of placental size by sonography shows that, similar to the GOS, the origins of macrosomia are present early in gestation (8). The risk of cephalopelvic disproportion has arisen because of the anatomical adaptations necessary for efficient bipedalism. These resulted in narrowing of the birth canal, imposing considerable constraints during parturition that are characteristic of modern humans even in comparison with neandertals

(9). The human head followed by the shoulders needs to rotate to fit the three planes of the pelvis that all have different shapes and orientations unlike other primates (10).

The combination of a narrow birth canal and the enlarged brain means that the Obstetric Dilemma is a particular problem for humans. Fetal growth in utero depends on development of a good maternal blood supply to the placenta that requires modification of the uterine spiral arteries. Trophoblast cells from the placenta invade deeply into the stroma to effect arterial conversion. The extravillous trophoblast cells (EVT) encircle the arteries and then cause direct destruction of the smooth muscle of the arterial wall with complete loss of vasoconstriction. In contrast, trophoblast invasion is much more limited in extent in Old World monkeys where trophoblast cells only move down the inside of the arteries to replace the endothelium and functionally modify the media (11,12). Amongst primates, deep trophoblast invasion through the decidua and into the inner myometrium is characteristic of human placentation, probably as a consequence of the increased blood supply needed to support the in utero development of the large brain. Indeed, brain development continues for some years after birth in humans (13). Thus, the extent of trophoblast invasion has a critical effect on fetal access to maternal oxygen and nutrients. Under-invasion is associated with GOS such as FGR and pre-eclampsia, a condition only occurring in humans; over-invasion can result in obstructed labour due to the large fetus. A consequence of the balancing selection operating to prevent obstructed labour and the birth weight from being too large is the persistence of pregnancies with pre-eclampsia and fetal growth restriction in all populations. The Obstetric Dilemma means that the birth weight must therefore be kept closely between the two dangerous extremes.

Regulation of placentation by the decidual immune system

Because trophoblast transformation of the arteries affects the growth and development of the fetus and placenta, there is much interest in how trophoblast invasion is controlled and thus how the supply line to the fetus is regulated. In vivo and in vitro observations show that human trophoblast cells are inherently highly invasive and can penetrate right through the wall of the uterus - *unless* they are controlled. This is seen, for example, after a caesarian section if the placenta implants on the scar in a subsequent pregnancy. Because the mucosal lining of the uterus (decidua) is missing at the site of the scar, trophoblast invasion proceeds unchecked, a condition known as placenta percreta, which can lead to uterine rupture (14). The same behaviour occurs in ectopic pregnancy where implantation occurs in the fallopian tube, which also lacks decidua. This suggests decidual tissue must be essential for regulation of trophoblast invasion.

To understand how decidua regulates placentation we have focused on the decidual immune system (15,16). Hints that the immune system was involved came initially from studying the epidemiology of pre-eclampsia (17). Classically, this is a disease of first pregnancies, giving rise to the idea that women became 'immune' afterwards. Change of male partners after a normal first pregnancy can trigger the condition; conversely women who have already had pre-eclampsia may then be protected when they have a baby with a new partner (18). These features, memory and specificity are characteristic of the immune system. The familial and genetic contribution from the mother is well established, but, importantly, several studies have also shown that there is a paternal contribution to both pre-eclampsia and birth weight (19,20,21). There is therefore circumstantial evidence that interactions between maternal and fetal immune system genes may determine successful pregnancy outcome.

There are two sites where fetal cells are in direct contact with maternal immune cells. Placental villi are covered by syncytiotrophoblast and present a large surface area in contact with maternal blood. Intermingling of maternal and fetal cells also occurs in the uterus at the site of placental

attachment where EVT invade into decidua Figure 1). A territorial boundary must be defined here between two individuals. Because of the need to regulate the blood flow to the placenta and thus the birth weight, this boundary needs to be established in the right place. If the placenta (fetus) is too intrusive, the mother is at risk of uterine rupture and death. If trophoblast invasion is not far enough, spiral arteries are not sufficiently transformed, the blood flow to placenta is reduced, fetal growth is compromised and the mother is at risk of pre-eclampsia. There is growing evidence for the role of the decidual immune system in the subtle delineation of this uterine maternal/fetal interface. Because these two individuals are genetically different, the invading trophoblast cells will express molecules encoded by paternal genes meaning that, in immunological terms, the fetus is 'non-self' to the mother. How do maternal immune cells recognize and respond to the fetal trophoblast cells in the decidua, and do these interactions regulate trophoblast invasion?

T cells and allorecognition in pregnancy

T cells recognise antigenic peptides presented by MHC molecules. These genes were originally discovered by Peter Medawar as the gene system whose products determine rejection or acceptance of transplants (22). Unlike somatic cells, syncytiotrophoblast lacks expression of both MHC class I and class II molecules, so cannot be recognized by T cells. However maternal T cell responses against fetal alloantigens do occur during pregnancy, generating fetal-specific T cells and humoral responses. (23). During pregnancy, temporary breaks in the synciotrophoblast layer can permit fetal cells to enter the maternal circulation. It is likely that these deported cells give rise to most of the fetal antigen-specific responses that have been described in T cells in maternal blood such as that to male HY minor histocompatibility antigen (24). There is no evidence that any of these responses can directly affect syncytiotrophoblast.

EVT in the decidua also express an unusual MHC profile: they lack class II expression and do not express the HLA-A or –B molecules normally found on

all somatic cells (25). Instead EVT express classical HLA-C as well as non-classical molecules, HLA-E and –G. The latter two are essentially monomorphic in human populations so will not vary from pregnancy to pregnancy (25). Since most T cells are restricted to recognize self HLA-A or-B molecules in association with a non-self peptide, the unusual MHC profile of EVT means that only HLA-C restricted T cells are capable of directly recognizing EVT in the decidua. A recent study has shown that in HLA-C mismatched pregnancies there are decidual T cells present that can recognize fetal HLA-C when stimulated with fetal cord blood. These are not present in HLA-C matched pregnancies. HLA-C mismatched pregnancies were also associated with an increase in CD4+CD25bright regulatory T cells (Tregs) in the decidua (26). Tregs represent a subset of CD4+ T cells that are act to suppress immune responses against self-antigens. They are characterised by expression of the transcription factor Foxp3 (27). However since EVT does not express MHC class II, these CD4+ Tregs presumably recognize fetal HLA-C through indirect allo-recognition via presentation of HLA-C peptides on maternal antigen presenting cells (APC). To date there is no evidence that T cells are present in human decidua that can directly recognize fetal alloantigens presented by HLA-C on trophoblast. However decidual Tregs are generated in HLA-C mismatched pregnancies and these may contribute to generation of a tolerogenic environment in the decidua.

Although there are significant differences in placental anatomy and trophoblast MHC expression compared to humans, elegant experiments possible in mice have given broadly similar results to those seen in humans. T cells specific for paternal antigens are generated in allogeneic matings, but do not lead to pregnancy failure in normal pregnancies (28). Adoptive transfer of CD8+ T cells specific for H2-K^b, to H2-K^b negative females carrying H2-K^b positive pups, did not result in pregnancy failure even though H2-$K^b$is expressed on trophoblast (29,30). However expansion of Tregs is seen during both syngeneic and allogeneic pregnancies and depletion of Tregs during pregnancy results in fetal resorption only in allogeneic matings (31). Tregs appear to play an important role in preventing rejection of

allogeneic pregnancies but the exact mechanism is unclear. In both mouse and humans multiple other mechanisms have been described that contribute to T cell tolerance. These include: 'trapping' of APCs within the decidua reducing migration to regional lymph nodes, reduced T cell entry into decidua by silencing of chemokine expression, stimulation of APCs by HLA-G to induce a tolerogenic cytokine secretion and many others (reviewed in 32,16). However, in humans there is no evidence that T cell dependent adaptive responses to fetal antigens, either systemically or in decidua, are involved in damaging placental cells or causing pregnancy failure.

Uterine NK cells and recognition of trophoblast HLA class I molecules

T cells comprise only 5-20% of decidual leukocytes. The overwhelming majority of leukocytes in the decidua are uterine Natural Killer cells (uNK). These are uniquely found in the endometrium during the luteal phase of the menstrual cycle when implantation occurs, and in decidua during early pregnancy (5). Uterine NK cells are phenotypically and functionally quite distinct from NK cells circulating in peripheral blood and do express a range of receptors that can bind molecules expressed by placental cells (33,34). Of these, our focus has been on those receptors that could be involved in specific recognition of paternal difference (5,16). We have therefore studied NK receptors that can bind the MHC molecules expressed by EVT: HLA-C, -E and –G. The latter two are essentially monomorphic in human populations so will not vary from pregnancy to pregnancy (25). Uterine NK cells express Killer Immunoglobulin-like Receptors (KIR) that can recognize HLA-C, and invading trophoblast cells abundantly express both maternal and paternal HLA-C molecules (35). However, the usual simple relationship between receptor and ligand is complicated by extreme polymorphism of both the KIR expressed by uNK and their trophoblast HLA-C ligands, with many different variants found within all populations. This means that there is a range of possible interactions between variants of maternal KIR and alleles of fetal HLA-C ligands to which uNK cells bind. Each pregnancy will thus bring together different combinations of maternal KIR and fetal HLA-C, with potentially different outcomes.

Killer Immunoglobulin-like Receptors (KIR) expressed by NK cells

The *KIR* gene family is complex with a wide range in both the number of *KIR* genes inherited by the mother as well as allelic variation of individual *KIR* genes (36). Up to 14 different *KIR* genes are present in a linear array in the Leucocyte Receptor Complex (LRC) on chromosome 19q13.4. KIR are major regulators of NK cell function with different KIR conferring either an inhibitory or activating signal to the NK cell. Their most important known ligands are HLA-C molecules. KIR are named based on the number of extracellular Ig-like domains (2D or 3D) and whether they have a long or short cytoplasmic tail (eg KIR2DL1 or KIR2DS1). Binding of KIR with a long cytoplasmic tail to their cognate ligands results in transduction of an inhibitory signal, whereas ligation of KIR with a short tail results in activation of NK cells. This complexity has been simplified by the clear distinction between two *KIR* haplotypes, *A* and *B*. The *KIR A* haplotype is the most common and contains six inhibitory genes in most individuals. The *KIR B* haplotype is much more variable in gene content and many of the additional KIR are activating. An individual's *KIR* genotype can thus be designated '*AA*' '*AB*' or '*BB*' (Figure 2). Although there are over 1000 alleles of HLA-C, these can be distinguished by KIR as two distinct groups, HLA-C1 and -C2, based on a dimorphism at position 80 of the ¤1 domain of the HLA-C molecule (36). To date there is evidence that five members of the KIR family are capable of binding to HLA-C allotypes. The known KIR specificities for HLA-C are shown in Figure 2. The strongest and most specific binding is of KIR2DL1 to HLA-C2 allotypes resulting in a strong inhibitory signal to NK cells.

Combinations of maternal KIR and paternal HLA-C genotypes regulate birth weight

To discover how this great polymorphism of maternal *KIR* and fetal *HLA-C* genes affects human reproductive success we have studied pregnancies where the *KIR* genotype of the mother and *HLA-C* group of her fetus is known. Our results show that there is a consistent relationship between

particular combinations of maternal *KIR* and fetal *HLA-C* ligands and birth weight (3,35,37,38). We compared maternal *KIR* and fetal *HLA-C* genotypes from a large number of first pregnancies selected from the Norwegian Mother-Baby cohort (MoBa) with known birth weight corrected for gestational age and fetal sex. A HLA-C2 group in the fetus is associated with pregnancies at the two extremes of birth weight especially when the *HLA-C2* allele is inherited from the father rather than the mother (3). If the mother has two *KIR A* haplotypes (*KIR AA* genotype), then she will have inherited two copies of the inhibitory *KIR* for HLA-C2 allotypes, namely *KIR2DL1*. Functionally, when uNK cells from a *KIR AA* woman bind trophoblast expressing a paternal HLA-C2 allotype this results in a strong inhibitory signal. In contrast, high birth weight pregnancies are associated with a paternally-derived *HLA-C2* allele, when the mother has inherited the activating *KIR* for HLA-C2, *KIR2DS1* that is located on the *KIR B* haplotype (3). This effect is quite significant; the average increase in birth weight in pregnancies with a fetus with paternal *HLA-C2* and *KIR2DS1* in the mother is ~200g, more than the differences found with sex of the baby (~50g) and high altitude (100g/1000 metres).

This immune interaction is not comparable to other situations such as organ transplantation where there is clear self/non-self discrimination because it is only *HLA-C* alleles carrying the C2 epitope that confer any effect. To date, in pregnancies where the fetus is homozygous for *HLA-C1* there is no influence of any *KIR* genotype on any clinical outcome in pregnancy that we have studied. This may be due to the tight specificity and strong inhibition conferred by KIR2DL1/HLA-C2 interactions compared to the weaker and more promiscuous KIR2DL2/3 interactions with HLA-C molecules (39). To summarize, at the site of placentation all the influences of *HLA-C* in pregnancy are mediated by paternal *HLA-C2* with the maternal *KIR* genotype determining the outcome (high or low birth weight). In pregnancies where the fetus is homozygous for *HLA-C1*, then the effect of the maternal *KIR* is neutral and no associations are seen (3,35).

Functions of uNK cells during placentation

The functional effects mediated by uNK cells at the uterine/placental interface relating to these different *KIR*/*HLA-C* combinations are hard to determine given the ethical and logistical problems of studying the site of placentation in early pregnancy in humans. The great disparity in placental strategies in different species also means animal models are generally uninformative. Furthermore, *KIR* genes are only a feature of simian primates, but even in these species there are fundamental differences in placentation with interstitial trophoblast invasion only a feature of the great apes (11). A further major difficulty has been the lack of any reproducible and reliable in vitro model systems to study human trophoblast function in vitro. Nonetheless, uterine NK cells can be isolated and, despite their name, are poorly cytotoxic (40,41). The input NK cells receive from KIR is either activating or inhibitory and NK function is a result of integrating this information and responding accordingly. When uterine NK cells receive an overall activating signal in a woman with a *KIR B* haplotype containing *KIR2DS1*, the output of soluble mediators like chemokines and cytokines (eg GM-CSF) is increased after co-culture with target cells bearing HLA-C2 molecules. This supernatant containing GM-CSF stimulates trophoblast invasion and migration in vitro (42). In contrast, when uNK cells from *KIR AA* women who only have the inhibitory KIR2DL1 for HLA-C2 were ligated with HLA-C2, trophoblast migration was not stimulated. This suggests there is reduced cytokine output by uNK cells in a woman with a *KIR AA* genotype when the overall signal is inhibitory. Thus, women with too much uNK cell inhibition (*KIR AA*) or too much activation (*KIR B* haplotype with KIR2DS1 present) are more likely to have babies with low or high birth weight respectively, when their uNK cells encounter trophoblast cells displaying a paternal HLA-C2 allotype. The interpretation we have made based on the genetic and functional evidence is that the overall uNK response determines how far the trophoblast moves into the uterus and modifies the arteries (Figure 3). Thus, there is a direct link between the function of the uterine immune system and the birth weight of the baby. This specialized immune interaction may be essential to maintain the fetal

maternal interface within the strict limits dictated by the Obstetric Dilemma.

Uterine NK cells also occur in the decidua in mice and appear to play an important role in vascular remodeling during placentation. Murine trophoblast invades into the decidua to a lesser degree than in humans and does not express non-classical MHC, so there are significant differences compared to human trophoblast (30). Although the major murine NK receptors that recognize MHC are the Ly49 family of receptors, they appear to function analogously to the human KIR. Mouse studies in which a single additional MHC molecule H2-D^d was introduced, showed impaired vascular remodeling and reduced fetal growth compared with identical mice lacking only H2-D^d (43). This MHC molecule binds the inhibitory receptor Ly49A and can inhibit additional uNK subsets when present. Particularly notable was the fact that reduced fetal growth was seen regardless of the parental origin of the H2-D^d molecule. These results confirm the idea that certain combinations of maternal NK receptors and paternal (or maternal) MHC molecules can influence trophoblast invasion and vascular remodeling. In both human and murine pregnancies, excessive inhibition of NK cell function is associated with reduced fetal growth.

Variation of *KIR* and *HLA-C* genes affects other diseases of pregnancy

There is other independent evidence that will substantiate this model in humans. In common with low birth weight, obstetric syndromes such as pre-eclampsia, and recurrent miscarriage are associated with reduced uterine invasion by trophoblast (44). Mothers at highest risk of these diseases are homozygous for the *KIR A* haplotype, and carry a fetus that has inherited *HLA-C2* from the father (35,37,38). Women with a *HLA-C2* fetus are protected from these disorders by the *KIR B* haplotype that contributes to protection in two possible ways. Firstly, many *KIR B* haplotypes include the activating KIR2DS1 that enhances trophoblast migration through enhanced secretion of 'pro-invasion' cytokines as described above. Secondly, the commonest *KIR2DL1* allele on the *B* haplotype is KIR2DL1*004. This binds

more weakly to HLA-C2 than KIR2DL1*003, the most common allele on the *A* haplotype (45). We predict that there would be reduced inhibition of uNK cells by KIR2DL1*004 on the *KIR B* haplotype. The fact that diverse GOS with a common underlying pathology of reduced trophoblast invasion, also exhibit a similar *KIR*/*HLA-C* risk profile, supports the idea that uNK/trophoblast interactions play an important role in all these pregnancy disorders.

If *KIR AA* and *HLA-C2* combinations are significantly disadvantageous in pregnancy, this raises the question as to why they have not been eliminated from human populations by natural selection. All populations have both *KIR A* and *KIR B* haplotypes and *HLA-C* alleles with both C1 and C2 epitopes, suggesting that a balance of *KIR A* and *B* haplotypes and their ligands is essential for the survival of a community (46). However different populations across the world have different frequencies of *KIR AA* genotypes and *HLA-C2* alleles. Evidence that there is strong selective pressure from reproduction comes from the inverse correlation between *KIR AA* and *HLA-C2* frequencies (37,47).

Pregnancy in African women

There are a few populations that do not fit this picture as they have high frequencies of both *KIR AA* genotypes and *HLA-C2* alleles. Notably these are all found in sub-Saharan Africa where high *HLA-C2* frequencies are characteristic. Sub-Saharan Africa is especially important in consideration of reproduction and birth, as maternal mortality rates are the highest in the world. Figures of 600/100,000 births compared with only ~15 in high-income countries mean this is one of the most extreme disparities in any health outcome (48,49). Obviously poverty and lack of access to medical care are major problems but there are several indications that pregnancy and parturition in women from sub-Saharan Africa have many different features compared with women in other parts of the world (50). Indeed, it appears that the Obstetric Dilemma is more of a problem for African women. Although there are no reliable records from Africa itself, studies of women

of recent African ancestry in Europe and the Americas all indicate that all the GOS (pre-eclampsia, stillbirth, FGR and prematurity) occur more commonly as would be predicted by the high prevalence of *HLA-C2* combined with *KIR AA* genotypes in these African populations (51). One explanation for maintenance of these 'reproductively risky' genotypes is the observation that an individual's resistance to infections such as hepatitis C virus is correlated with a *KIR AA* genotype and *HLA-C1* (52). A simple model would be that human populations must maintain the *KIR A* haplotype and *HLA-C1* in order to ensure resistance to pathogens. In contrast, reproductive success and the development of the larger human brain and more robust progeny are dependent on maintenance of *KIR B* haplotypes and *HLA-C2*. In each human population the relative frequencies of these haplotypes will be subject to natural selection depending on pathogen load, reproductive pressure and of course will change of time (12). The fact that all human populations maintain both *KIR A* and *B* haplotypes as well as *HLA-C1* and *-C2*, suggests that populations that lose one of these components are doomed to extinction when subjected to extreme selection by events such as infection, famine and warfare.

While the factors responsible for the high incidence of *HLA-C2* and the *KIR A* haplotype in current African populations are not yet clear, this undoubtedly contributes to the high frequency of pre-eclampsia, stillbirth and fetal growth restriction found in sub-Saharan Africa. It is interesting to note that in such populations there is also an increased frequency of obstructed labour (50). This may be connected to the bony pelvis as measurements obtained by pelvimetry indicate that in African women some aspects of the birth canal are smaller (53). A high frequency of *HLA-C2* and the *KIR A* haplotype may therefore reflect selection to reduce the birth weight but this will also result in an increased risk of the GOS. In keeping with this, the development of the fetal organs is accelerated and the gestational age is shorter at 38 compared to 40 weeks (50,54). This clearly illustrates how the complex selective pressures acting on the evolution of the KIR/HLA-C

system could regulate human reproductive fitness, brain size, pathogen resistance and even the rate of fetal development.

Conclusion

The adaptations of the pelvis associated with bipedlaism coupled with the larger human brain, severely limit human birth weight. Conversely neonatal survival of smaller babies is greatly reduced. Human birth weight is thus subject to stabilizing selection. Although the control of birth weight is complex, adequate placentation to permit access to nutrients in maternal blood is essential for normal fetal growth. In humans this is achieved by trophoblast cells, which remodel maternal spiral arteries. Inadequate trophoblast invasion is associated with obstetric syndromes including pre-eclampsia, recurrent miscarriage and FGR. We have shown that recognition of HLA-C on invading trophoblast by KIR receptors on maternal uNK plays a role in controlling trophoblast invasion and hence birth weight. Because both *KIR* and *HLA-C* genes are highly polymorphic, each pregnancy will bring together different combinations of maternal activating or inhibitory KIR that can recognize fetal HLA-C. Our findings suggest that binding of strongly inhibitory KIR to HLA-C2 expressed on trophoblast is detrimental to placentation. This combination is found more frequently in small babies. The presence of maternal activating receptor *KIR2DS1* together with fetal *HLA-C2* is associated with increased birth weight, suggesting the activation of uNK favours placentation. The association is strongest when a fetal *HLA-C2* allele is inherited from the father. These findings suggest that a balance of maternal activating and inhibitory *KIR* and *HLA-C2* genotype frequencies act to maintain birth weight at an optimum for maternal and fetal survival.

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Figure Legends

Figure 1.

Fetal growth restriction is associated with reduced remodelling of maternal spiral arteries by trophoblast cells. In normal pregnancy (right hand panel), extravillous trophoblast cells migrate as far as the myometrium and also infiltrate into the arterial media and endothelium of maternal spiral arteries. This results in dilatation and increased flow of maternal blood at low pressure into the intervillous space (red arrow). In pregnancies affected by pre-eclampsia or FGR (left panel), the depth of trophoblast invasion is reduced with less spiral artery remodelling. Blood flows at higher pressure and is more pulsatile, resulting in placental stress, reduced placental development and poor fetal growth.
(Figure adapted from Moffett-King, 2002, reference 5, with permission).

Figure 2.

Representative *KIR A* and *B* haplotypes of the *KIR* gene family, together with their known HLA-C binding specificities (C1 and C2) depicted above their cognate KIR receptors. KIR2DS4 binds a limited number of C1 and C2 allotypes. Activating *KIR* are shown as blue boxes, inhibitory *KIR* are shown in red. Framework *KIR* genes that are present in all haplotypes are shown in black. Note many of the *KIR* shown on the *B* haplotype are not present in all individuals.

Figure 3.

Presence of maternal *KIR2DS1* is associated with increased birth weight.

A. Distribution of birth weights in the Norwegian MoBa cohort correlates with increased neonatal morbidity (frequency of transfer to special care baby unit) at the extremes of birth weight (3). The cohort was divided into low (<5th centile) normal (6-89th centile) and high (>90th centile) birth weight babies. The frequency of maternal *KIR AA* genotype + fetal *HLA-C2* is increased in the small babies. Presence of maternal *KIR2DS1* and fetal *HLA-C2* is associated with increased birth weight.

B. Proposed model for maternal KIR/fetal HLA-C interactions at the site of placentation. In this model the fetus has inherited a paternal *HLA-C2* allele and is homozygous for *HLA-C2*. If the mother has a *KIR AA* genotype, then KIR2DL1 binds strongly to trophoblast HLA-C2 molecules resulting in strong inhibition of uNK cells. This is associated with defective placentation. In contrast, when the mother has a *KIR AB* or *BB* genotype, the KIR2DL1 alleles on the B haplotype tend to inhibit uNK function more weakly when they bind C2. The *KIR B* haplotypes also contain the activating *KIR2DS1*. In this situation uNK cells are stimulated to produce increased levels of cytokines such as GM-CSF that can enhance placentation.

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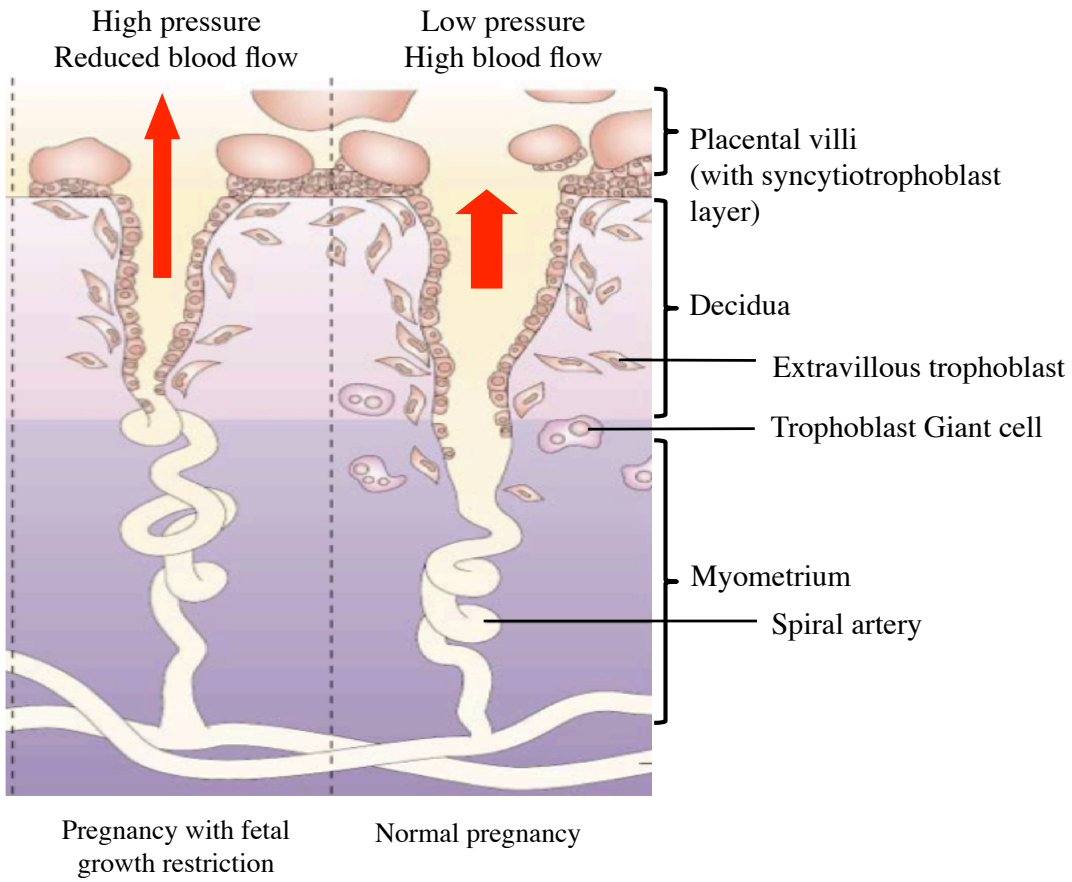
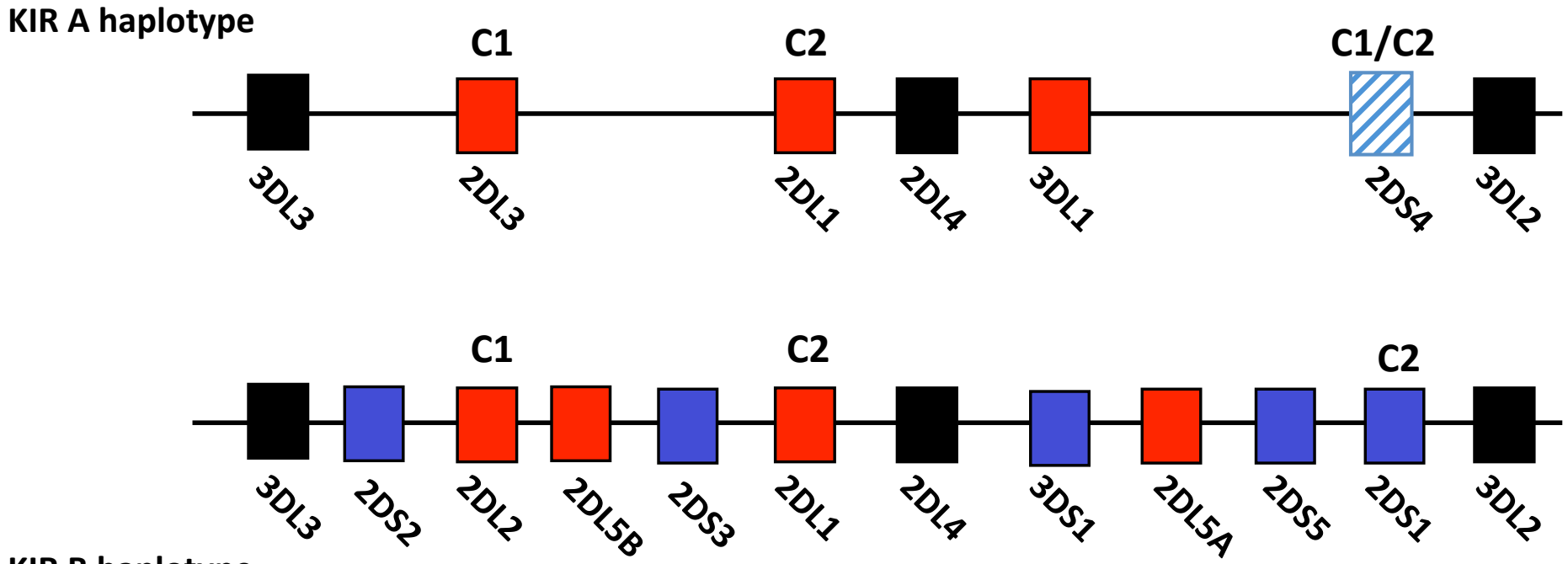

High pressure
Reduced blood flow
Low pressure
High blood flow
Placental villi
(with syncytiotrophoblast
layer)
Decidua
Extravillous trophoblast
Trophoblast Giant cell
Myometrium
Spiral artery
Pregnancy with fetal
growth restriction
Normal pregnancy


KIR A haplotype
C1
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KIR B haplotype
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3DL3
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2DL5B
2DS3
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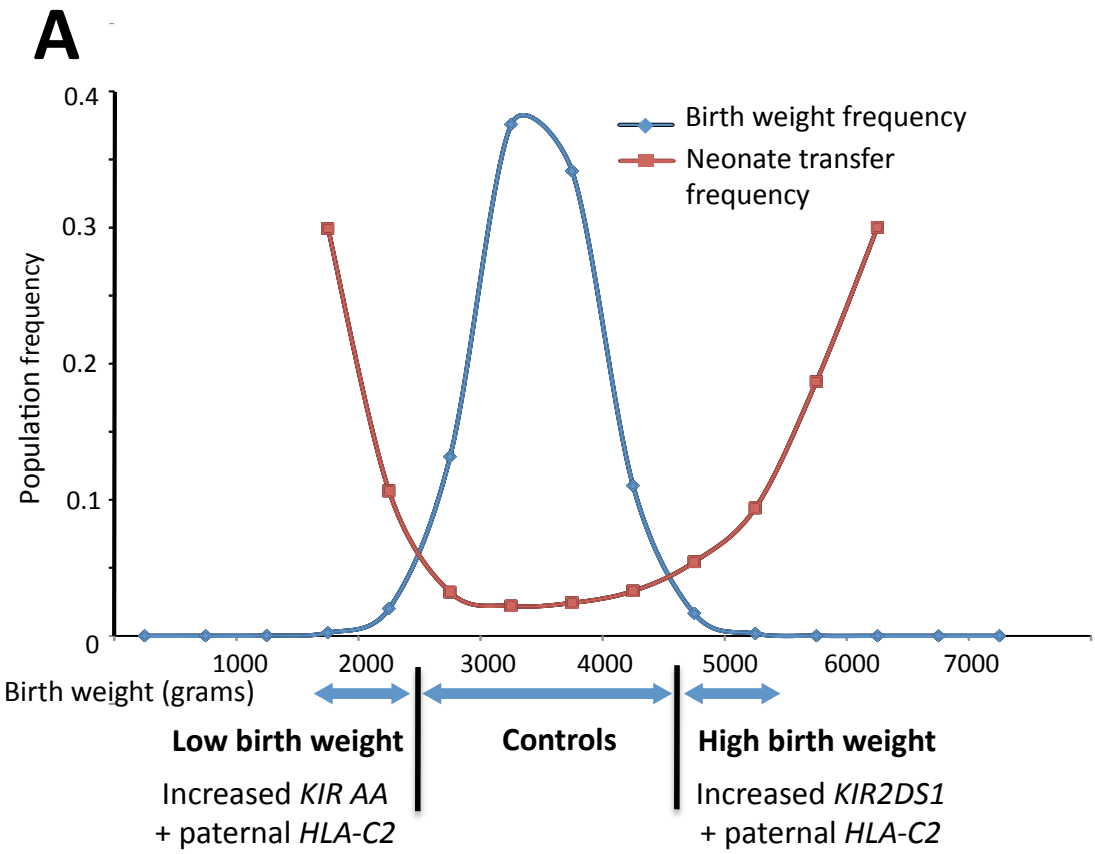
Framework gene
Activating KIR
Inhibitory KIR
>60% of individuals lack full length form of activating KIR2DS4


A
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0.3
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Population frequency
Birth weight frequency
Neonate transfer frequency
1000
2000
3000
4000
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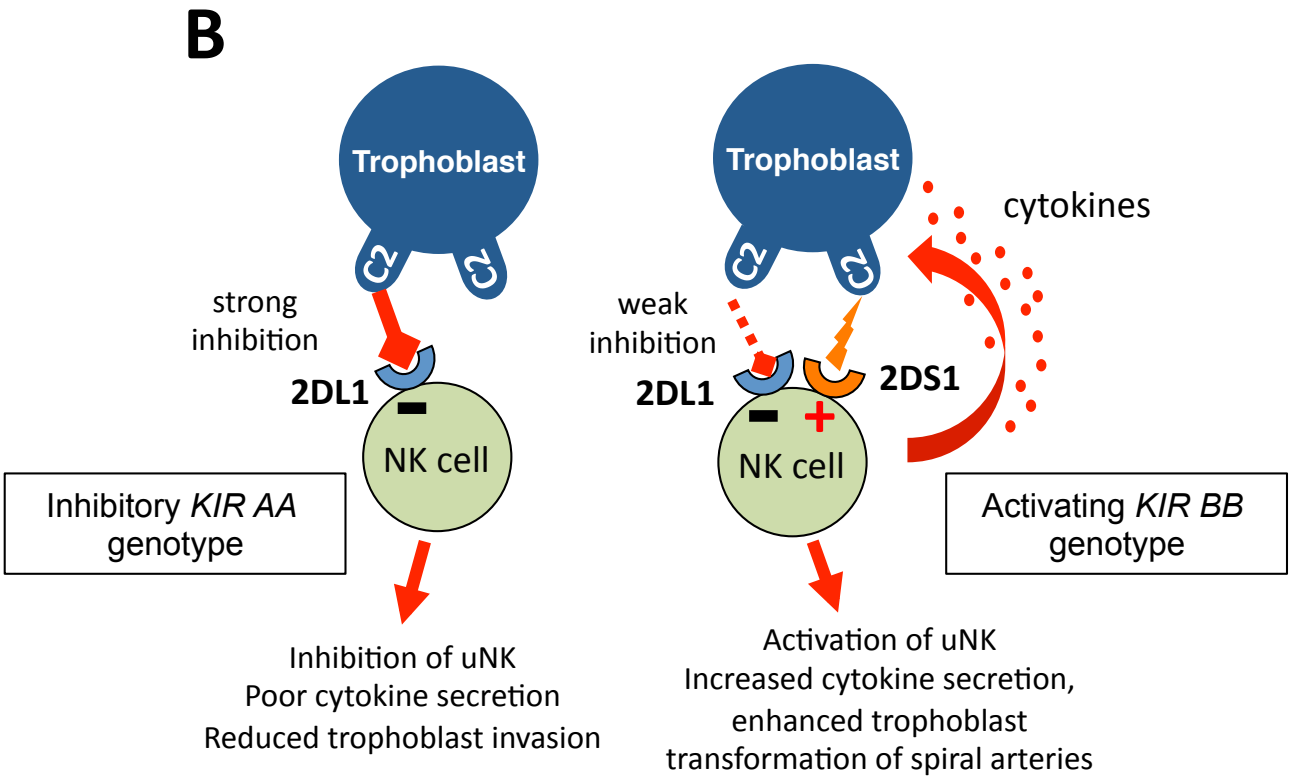
7000
Birth weight (grams)
Low birth weight
Increased KIR AA
+ paternal HLA-C2
Controls
High birth weight
Increased KIR2DS1
+ paternal HLA-C2


B
Trophoblast
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Poor cytokine secretion
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weak
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NK cell
Activating KIR BB
genotype
Activation of uNK
Increased cytokine secretion,
enhanced trophoblast
transformation of spiral arteries