In Sub-Saharan Africans, maternal mortality is unacceptably high with >400 deaths/100,000 births compared to <10/100,000 in Europeans. One third of the deaths are caused by pre-eclampsia, a syndrome arising from defective placentation. Controlling placentation is maternal natural killer cells that use killer-cell immunoglobulin-like receptors (KIR) to recognize the fetal HLA-C molecules on invading trophoblast. We analysed genetic polymorphisms of maternal KIR and fetal HLA-C for 484 normal and 254 pre-eclamptic pregnancies at Mulago Hospital, Kampala, Uganda. The combination of maternal KIR AA and fetal HLA-C2 associates with pre-eclampsia (P=0.0318, OR 1.49). The KIR genes associated with protection are located in centromeric KIR B regions that are unique to Sub-Saharan African populations and contain the KIR2DS5 and KIR2DL1 genes (P=0.0095, OR 0.59). By contrast, telomeric KIR B genes protect Europeans against pre-eclampsia. Thus, different KIR B regions protect Sub-Saharan Africans and Europeans from pre-eclampsia, whereas in both populations the KIR AA genotype is a risk factor for the syndrome. These results emphasize the importance of undertaking genetic studies of pregnancy disorders in African populations with the potential to provide biological insights not available from studies restricted to European populations.

Pre-eclampsia is especially common in African women, and is a major cause of maternal death. The KIR genes we analyzed are carried by Natural Killer cells, immune cells that populate the uterus and are essential for successful pregnancy. KIR proteins bind HLA ligands on the implanting placental trophoblast cells. African and European women share similar risk associations for pre-eclampsia, but protection is associated with different KIR genes in the two populations. African women are protected by a combination of KIR B haplotype genes that is present almost exclusively in Africans. This study emphasizes the importance of studying diseases in Africans where the KIR/HLA genetic system is at its most diverse and maternal mortality rates are the highest in the world.

Significance

Pre-eclampsia is especially common in African women, and is a major cause of maternal death. The KIR genes we analyzed are carried by Natural Killer cells, immune cells that populate the uterus and are essential for successful pregnancy. KIR proteins bind HLA ligands on the implanting placental trophoblast cells. African and European women share similar risk associations for pre-eclampsia, but protection is associated with different KIR genes in the two populations. African women are protected by a combination of KIR B haplotype genes that is present almost exclusively in Africans. This study emphasizes the importance of studying diseases in Africans where the KIR/HLA genetic system is at its most diverse and maternal mortality rates are the highest in the world.
Table 1. Frequency of maternal KIR genotypes and KIR gene carriers

<table>
<thead>
<tr>
<th>KIR GENOTYPE</th>
<th>UK Pre-eclampsia cases (n=729) n (%)</th>
<th>UK Controls (n=592) n (%)</th>
<th>OR (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR AA</td>
<td>266 (36.5)</td>
<td>163 (27.5)</td>
<td>1.51</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>(1.05-2.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR AB</td>
<td>456 (62.6)</td>
<td>424 (71.6)</td>
<td>0.59</td>
<td>(0.43-0.81)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR BB</td>
<td>7 (0.96)</td>
<td>5 (0.84)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KIR GENES

| 2D5       | 247 (98.4)                          | 474 (98.1)                | NS      |         |
| 2DL1      | 247 (98.4)                          | 476 (98.6)                | NS      |         |
| 2DL2      | 132 (52.6)                          | 293 (60.7)                | 0.0365  |         |
| 2DS3      | 222 (88.4)                          | 414 (85.7)                | NS      |         |
| 2DS5      | 138 (55.0)                          | 316 (65.4)                | 0.0061  |         |

Fig. 1. Frequency of the KIR AA genotype alone and in combination with the fetal HLA-C carrier group in Uganda and in the UK. The frequency of KIR AA genotypes shown when combined either with a fetus carrying a C2 epitope or those lacking C2 and carrying only C1-bearing HLA-Callotopes. There is a significant risk of pre-eclampsia when the KIR AA genotype is shown when combined either with a fetus carrying a C2 epitope or those lacking C2 and carrying only C1-bearing HLA-Callotopes. There is a significant risk of pre-eclampsia when a KIR AA women has a fetus carrying a C2 epitope for both cohorts; in Uganda *P=0.0318, OR 1.49, in the UK *P=0.0267, OR 1.46.

Results

Clinical characteristics of the cohort. This case-control study of pre-eclampsia involved 738 pregnant women at Mulago Hospital, Kampala in Uganda. More than 90% of cases and controls were Bantu, the largest ethnic group, with small numbers of Luo, Nilo-Hamites and other ethnic groups. The ethnicity of the male partners and the sex ratios of the singleton babies in all the groups were similar (Table S1). HIV+ women were not excluded from the analysis as there were similar numbers in both pre-eclampsic and control pregnancies (~5%) (Table S1) and similar results were found even when HIV+ women were omitted (Table S2). As expected, the gestational age at delivery and the birth weights were significantly lower in the pre-eclampsic cases compared to controls (P<0.001, Table S1, Figure S1). Unlike European women, KIR B centromeric regions containing KIR2DS5 protect Ugandan women from pre-eclampsia.
Parameter | P-value* | OR (CI)
--- | --- | ---
Effect of relative dose of maternal and fetal HLA-C2 alleles | | 
Fetus had fewer C2 alleles than the mother | 0.0705 | 1.087 (0.69-1.69)
Fetus had the same number of C2 alleles | 0.1612 | 1.280 (0.91-1.80)
Fetus had more C2 alleles than the mother | 0.0130 | 1.724 (1.12-2.64)
Effect of origin of fetal HLA-C2 allele | | 
Paternal origin | 0.0203 | 1.795 (1.10-2.93)
Maternal origin | 0.5222 | 1.162 (0.72-1.84)

*Fisher’s exact test with mid-p adjustment

Maternal KIR AA genotype is increased in the pre-eclamptic pregnancies (P=0.0256, OR 1.45)(Table 1), particularly when combined with the presence of fetal HLA-C alleles encoding the C2 epitope, similar to our findings in Europeans (P=0.0318, OR 1.49)(Figure 1). We then analyzed which KIR B haplotype genes are protective. Three KIR B genes, KIR2DL2, KIR2DL5 and KIR2DS5, are more frequent in controls than in women with pre-eclampsia. Of these three, only KIR2DS5 is significantly protective for women with pre-eclampsia after Bonferroni correction (P=0.0009, P=0.0126, OR 0.59)(Table 1)(Figure 2A) (Table 3). In comparable studies on European women, protection was seen with KIR2DS1 and not with KIR2DS5 as shown here for African women (Table 1). Moreover, in the Ugandans, the telomeric B (IB) genes KIR2DS1 and KIR2DS3 are at similar low frequency in cases and controls (Table 1).

As KIR genes are in linkage disequilibrium, KIR2DS5 could be itself protective or marking a nearby protective gene. KIR2DS5 can be found in both the KIR centromeric B (cB) and telomeric B (IB) regions. To determine the location of KIR2DS5 in our cohort, we grouped individual genotypes according to their combination of centromeric and telomeric KIR regions, based on previously described African KIR haplotypes (see Methods and Figure 3). Genotypes characteristic of expanded or contracted regions were also identified and shown to have similar frequencies in cases and controls.

Next, allele-level KIR2DS5 typing was performed, which identified ten alleles that were assigned to cB or IB regions as described in Methods (Figure 4). KIR2DS5*004, *006, *007, and *010 are restricted to cB, whilst KIR2DS5*002, *003, *008, and *011 are restricted to IB. KIR2DS5*005 is the most frequent allele and the only one found in both cB and IB (Figure 4), pointing to it being the progenitor of all other KIR2DS5 alleles. Our assignments of KIR2DS5 alleles to cB or IB agree with those defined by complete KIR haplotype sequences and analysis of African and African-American families (15, 18, 19). With all this information, we were able to determine the centromeric or telomeric location of KIR2DS5 for all KIR2DS5-carrying individuals.

Comparison of the frequency of the centromeric and telomeric KIR2DS5 alleles in cases and controls shows that they differ in the protection they provide against pre-eclampsia. KIR2DS5 is protective in Ugandan women when it is present in the cB region (cB01 or cB03, P=0.0005, OR 0.59) (Figure 2B, Figure 3, Table S3). Furthermore, of all the cB KIR2DS5 alleles, only KIR2DS5*006 is significantly more frequent in controls than in pre-eclamptic pregnancies (P=0.0015, OR 0.35) (Figure 2C, Table S4). The dominant allele, KIR2DS5*005, has similar frequencies in both cases and controls even when we can unequivocally assign its location to cB and thus appears neutral. Consistent with the low frequency of KIR2DS1 and KIR3DS1 in Africans, KIR2DS5 is less frequently present in IB than cB. When present in IB it has no effect, being at similar frequencies in controls and cases (Figure 2B, Table S3). Thus, the protective effect of KIR B is not just the absence of KIR A genes but also the presence of genes belonging to a particular subgroup of cB regions, cB01 or cB03 (Figure 3).

In Ugandan women, like European women, pre-eclampsia associates with maternal KIR A4 genotype combined with fetal expression of paternal HLA-C2. We further examined the effect of different combinations of maternal KIR and fetal ligands, C1 and C2 epitopes of HLA-C alleles. Considered alone, the C1 and C2 frequencies in mothers and babies do not significantly differ between cases and controls (Table S5). Using an extended Mantel-Haenszel test for linear trend, we find that KIR AB or BB genotype mothers carrying a C1C1 homozygous fetus have the least risk of pre-eclampsia, whereas a KIR AA mother carrying a C2 fetus has greatest risk (P=0.0122) (Figure S2, Table S6). Other genetic combinations have risks between these two extremes.

If the fetus has one more HLA-C allele encoding a C2 epitope than the mother, then the fetus must have inherited this C2 from the father. In this situation, the risk of pre-eclampsia in the absence of KIR2DS5 is increased (P=0.0130, OR 1.72) (Table 2). To explore this further we defined the parental origin of the C2 for C1C2 heterozygous fetuses. When the single C2 is paternally inherited the risk of pre-eclampsia associated with the absence of KIR2DS5 is greater (P=0.0203, OR 1.80) than when it is maternally inherited (NS, OR 1.16 CI 0.72-1.84) (Table 2). Taken together, these findings show that there is an increased risk of pre-eclampsia in women with a KIR AA genotype lacking...
Recurrence of pre-eclampsia in Ugandan women is associated with maternal KIR AA genotype and fetal expression of paternal C2. The risk of recurrence of pre-eclampsia is known to be high (~20%) (20, 21). In our cohort were 24 pre-eclamptic women who had recurrence of a hypertensive disorder of pregnancy, a condition on the same spectrum as pre-eclampsia. The 45.8% frequency of the KIR AA genotype in these women with recurrent pre-eclampsia was even higher than the frequencies of 36.3% in the full cohort and 28.2% in controls. Ten of the eleven KIR A4 pregnancies in this sub-cohort carried a C2 fetus.

**Discussion**

Our genetic study in an African population not only supports previous findings that certain combinations of maternal KIR and fetal HLA-C variants are associated with pre-eclampsia but also reveals the benefits of studying multiple populations including those most at risk of a disease. Pre-eclampsia occurs more commonly in African women and the symptoms are of severe, early onset disease associated with low birth weight and high mortality (4). Our findings have relevance to other disorders of pregnancy as unexplained stillbirth, fetal growth restriction and preterm labour are more common women with African ancestry and share the same underlying problem of defective placentation with reduced maternal blood flow to the placenta (4).

There is considerably more genetic diversity of KIR genes in Africans both at the level of KIR haplotypes and number of alleles at individual KIR loci (10, 15, 16). Despite this complexity, we find complete consistency with our studies of pre-eclampsia in Europeans: the risk is associated with a maternal KIR A4 genotype combined with a paternally-derived HLA-C allele carrying a C2 epitope in the fetus (8, 17). Recurrent pre-eclampsia frequently occurs in African women (24.6% in a recent Tanzanian study) and the high frequency of KIR A4 genotypes in these women in our study is striking (45.8% compared to 28.2% in controls)(21). The KIR always present on the KIR A haplotype likely confer this risk for is KIR2DL1, an inhibitory KIR with strict specificity for C2 epitopes (22). Thus, in women with a KIR A4 genotype containing two copies of KIR2DL1, uNK will be strongly inhibited when confronted by HLA-C2+ trophoblast. There are at least 12 KIR2DL1 alleles located in A1 region in Africans compared to 1-5 in other populations (15). In the future analysis of larger cohorts, including more women with recurrent pre-eclampsia, should identify if there are particular KIR2DL1 alleles responsible.

One clear difference that might partially explain the increased risk of pre-eclampsia in Africans is the higher frequency of C2-bearing HLA-C allotypes across SSA compared with elsewhere in the world (14). The probability of African women having a C2-positive partner or fetus is 80% compared to 64% for European women. Similarly the probability of African women having a fetus carrying a paternal C2 epitope is 55%, compared to 40% for European women (Table S4). Given the selective pressure that pre-eclampsia imposes on a population, there must be other scenarios where C2 epitopes are beneficial. HLA-C and KIR are immune system genes with roles in outcome from viral infections such as HCV and HIV (10, 23-25). In SSA C2 epitopes might be advantageous in responding to a range of pathogens, including malaria. Studies of how HLA-C and KIR variants affect responses to infection in SSA are still limited, especially in the crucial period from birth to adolescence.

We observed that B regions containing KIR2DS1 provide a protective effect for pre-eclampsia in Europeans (8). In contrast, we now show that in Ugandans KIR Cb regions characterized by KIR2DS5, KIR2DP1 and KIR2DL1 (cB01 and cB03) are protective. The low carrier frequency of KIR2DS1 in SSA (1.4%–27.8%) compared to Europe (42.5%) also suggests that KIR2DS1 does not play an important role in pregnancy success in Africans (14). One explanation for the different protective effect is that KIR2DS5, an activating KIR that likely evolved from a KIR specific for C2, does function like KIR2DS1- although there is no evidence to date that the C2 epitope is a KIR2DS5 ligand (22). The single KIR2DS3 allele in Europeans, KIR2DS3*002, is in tight LD with KIR2DS1 and located in the Bb region. Unlike Europeans though, KIR2DS5 is polymorphic in Africans and African-Americans. We found 10 alleles in Ugandans, consistent with previous reports from African Americans.
common in African women with associated features of pregnancy that favour smaller babies: earlier birth - the gestational age is reduced to 38 weeks, the head engages late into the pelvis and the baby matures earlier than in non-Africans (4). Thus, there is not only high mortality in mother and babies from pre-eclampsia (associated with low birth weight and still birth), but also at the other end of the normal birth weight spectrum. Both mothers and their babies benefit if the latter have intermediate birth weights and the two extremes of very low and high birth weight are selected against. The balance between these two extremes is partially determined at placentation when uNK allow trophoblast cells to access sufficient maternal oxygen and nutrients without starving the baby (defective trophoblast invasion) or risking uterine rupture (excessive trophoblast invasion) (3). In an African population, because of the greater risk of cephalo-pelvic disproportion (4), there is even greater selection for reduced fetal size with associated pre-eclampsia - this is consistent with the higher frequency of maternal KIR AA/paternal C2 combinations in SSA.

In Europeans, opposing KIR/HLA-C combinations are associated with the extremes of birth weight: a paternal C2 epitope is associated with both extremely low birthweight and high birth weight (<5th centile) the risk is with maternal KIR AA genotype, whilst in high birth weight the association is with maternal KIR2DS1 (35). Studies on how these genetic findings are translated in uNK functional differences are still limited but we found that when KIR2DS1+ uNK (isolated from UK patients) are activated by target cells expressing HLA-C2, there is increased production of soluble factors (eg GM-CSF) that enhance trophoblast invasion (34).

Thus, there is a balance between the KIR A and KIR B haplotypes in both populations but they differ in the regions of the KIR B haplotype that correlate with protection from pre-eclampsia. 

Materials and Methods

Ethics statement. Approval to conduct the study was given by the Higher Degrees Research and Ethics Committee of Makerere University College of Health Sciences and the Uganda National Council for Science and Technology (UNCST). The participants gave written informed consent to participate in the study. Withdrawal from the study never jeopardized health care and this was provided free to all women.

Study design. This study was conducted at Mulago National Referral and Teaching Hospital, located in Kampala, which functions as a tertiary referral center for Uganda. Mulago hospital is the busiest maternity hospital in Sub-Saharan Africa, with over 80,000 births a year. Genomic DNA was obtained from maternal blood from unrelated healthy women (n=484) or women with pre-eclampsia or eclampsia (n=254) between July 2009 and June 2011. Umbilical cord samples were obtained from the babies for genomic DNA isolation. Pre-eclampsia was defined as hypertension of 140/90 mmHg or more, on more than one occasion at least 4 hours apart plus proteinuria of +2 or more by dipstick, both at 20 weeks or more of gestation. Eclampsia was defined as preeclampsia plus convulsions. Controls were women with a normal first pregnancy delivering at term (≥38 weeks) who were normotensive with no proteinuria. Excluded from controls were patients taking long term medication and patients with other diseases including chronic hypertension and renal disease but excluding HIV. Women who had received a blood transfusion within the last 3 months were also excluded. Cases and controls were consecutively recruited from the same catchment area during the study period. Data was collected...
DNA isolation and genotyping. Maternal genomic DNA was isolated from 5 ml of blood using the QIAamp DNA Maxi Blood Kit (Qiagen). Fetal DNA was isolated from umbilical cord samples after overnight incubation with Proteinase K (Roche), purification with a protein precipitation solution (Qiagen) followed by ethanol precipitation. Twelve maternal KIR genes were typed for presence or absence by PCR-SSP using two pairs of primers per gene as described previously (16, 17, 18). The KIR genes typed were 2DL1, 2DL2/3, 2DL5, 3DL1/S1, 2DP1, 2DS1, 2DS2, 2DS3, 2DS4 (including the deletion), and 2DS5. All the samples were typed for KIR2DL1 and KIR2DP1 copy number. Me and selected samples were further investigated for additional KIR (2DL4, 3DP1, 3DL3, 3DL5) so that all 14 KIR genes were included (19). Individual genotypes were defined according to their combination of centromeric (LA and CB) and telomeric (LA and CB) KIR regions, based on previously described African KIR haplotypes (14, 15, 18). We first discriminated KIR A from KIR B regions on the basis of the presence/absence of 2DS2, 2DL23, 2DL1, 3DL1, 3DS1, 2DS5 and 2DS4. There are common CB regions in Africans (Figure 3) that were identified in individuals with a CB region using information from the presence/absence of individual KIR genes and the copy number of KIR2DL1 and KIR2DP1 (18). Typically, CB01 and CB03 have common haplotypes, reproduction and human evolution. Nat Rev Immunol 13(13):133-144. KIR2DS alleles were genotyped by pyrosequencing, targeting exons 5, 6, and 7 (15). Then, by knowing which KIR2DS alleles are present in individuals homozygous for either centromeric (LA or CB) or telomeric (A or CB) regions, we could assign each of the ten KIR2DS5 alleles to CB or CB (Figure 4). C1 and C2 were defined in maternal and fetal samples based on the primers and methods described previously (8, 16). HLA-C low resolution allelic typing was performed using a PCR-SSP method consisting of 21 reaction wells adapted from (18). Each well contained a final reaction volume of 10µl, consisting of SY Green (Roche), Buffer (Promega), 0.2mM dNTPs (ThermoFisher), 1.25mM MgCl2 (Promega), 0.4U GoTaq DNA polymerase (Promega), 134mM 66/64 control primer (Eurogentec) and approximately 45ng DNA. PCR products were run on a 1% agarose gel and visualized using a UV and ethidium bromide.

Statistical analysis. Unless otherwise indicated, categorical data was analyzed using the chi-square and Fischer’s exact test with two-tailed mid p adjustment and Student’s t-test for continuous data. A P value of ≤0.05 was considered to be statistically significant. The magnitude of the effect was estimated by odds ratios (OR) and their 95% confidence intervals (CI).

ACKNOWLEDGEMENTS. We thank all the patients, the midwives and the laboratory staff. In particular, Margaret Sewagaba, Florence Mugema, Dorothy Mugabi, Anastazia Karungi, and Prossy Namukwasa. This work was supported by the Wellcome Trust (090108/Z/09/Z, 085992/Z/08/Z, 089821/Z/09/2), the British Heart Foundation (PG/09/077/2964), the Centre for Trophoblast Research at the University of Cambridge, a Wellcome Trust Uganda PhD Fellowship in Infection and Immunity held by Annettee Nakimuli, funded by a Wellcome Trust Strategic Award (084344), the US National Institutes of Health (AI107982), and the UK Medical Research Council (G0091682).

2. Trophoblast Research at the University of Cambridge, a Wellcome Trust Uganda PhD Fellowship in Infection and Immunity held by Annettee Nakimuli, funded by a Wellcome Trust Strategic Award (084344), the US National Institutes of Health (AI107982), and the UK Medical Research Council (G0091682).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-eclampsia cases</th>
<th>Controls</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=254</td>
<td>n=484</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Women’s age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>24.8 ± 5.4</td>
<td>21.1 ± 2.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>range</td>
<td>(13.0-42.4)</td>
<td>(16.0-31.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravidae</td>
<td>132 (52.0)</td>
<td>484 (100)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Multiparous</td>
<td>122 (48.0)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Sex of baby</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>109 (46.0)</td>
<td>248 (51.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>128 (54.0)</td>
<td>236 (48.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Gestation age at delivery (weeks)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>37.2 ± 3.7</td>
<td>39.8 ± 1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>range</td>
<td>(24-44)</td>
<td>(38-45)</td>
<td></td>
</tr>
<tr>
<td><strong>Baby’s birth weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>2.6 ± 0.8</td>
<td>3.1 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>range</td>
<td>(0.7-4.5)</td>
<td>(2.0-4.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Admission BP– systolic (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>163.8 ± 21.7</td>
<td>110.5 ± 7.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>range</td>
<td>(140-254)</td>
<td>(90-135)</td>
<td></td>
</tr>
<tr>
<td><strong>Admission BP– diastolic (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>110.2 ± 14.8</td>
<td>67.2 ± 6.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>range</td>
<td>(90-160)</td>
<td>(60-85)</td>
<td></td>
</tr>
<tr>
<td><strong>HIV status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>241(94.9)</td>
<td>461 (95.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Positive</td>
<td>13 (5.1)</td>
<td>23 (4.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher’s exact test with mid-p adjustment
### Table S2 Summary of the results for HIV negative individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-value*</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal KIR AA</td>
<td>0.0167</td>
<td>1.50 (1.08-2.10)</td>
</tr>
<tr>
<td>Maternal KIR AA and fetal HLA-C2+</td>
<td>0.0318</td>
<td>1.49 (1.04-2.15)</td>
</tr>
<tr>
<td>Presence of KIR2DL2</td>
<td>0.0179</td>
<td>0.68 (0.50-0.94)</td>
</tr>
<tr>
<td>Presence of KIR2DL5</td>
<td>0.0047</td>
<td>0.63 (0.46-0.87)</td>
</tr>
<tr>
<td>Presence of KIR2DS5</td>
<td>0.0009</td>
<td>0.58 (0.42-0.80)</td>
</tr>
<tr>
<td>Fetus had more HLA-C2 alleles than the mother</td>
<td>0.0083</td>
<td>1.81 (1.17-2.79)</td>
</tr>
<tr>
<td>Paternal origin of fetal HLA-C2 allele</td>
<td>0.0256</td>
<td>1.82 (1.08-3.07)</td>
</tr>
<tr>
<td>Presence of KIR2DS5 centromeric</td>
<td>0.0089</td>
<td>0.58 (0.38-0.88)</td>
</tr>
<tr>
<td>Presence of KIR2DS5*006</td>
<td>0.0034</td>
<td>0.38 (0.18-0.74)</td>
</tr>
<tr>
<td>Trend of maternal KIR and fetal HLA-C combinations</td>
<td>0.0086†</td>
<td>NA</td>
</tr>
</tbody>
</table>

Pre-eclampsia cases (n=238), controls (n=460), all HIV negative

*Fisher's exact test with mid-p adjustment unless otherwise stated

†Extended Mantel-Haenszel chi square for linear trend

NA not available
Table S3 Frequency of the different *KIR2DS5* genotypes

<table>
<thead>
<tr>
<th>KIR2DS5 genotype</th>
<th>Pre-eclampsia cases (n=251) n (%)</th>
<th>Controls (n=483) n (%)</th>
<th>P-value*</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of <em>KIR2DS5</em></td>
<td>157 (62.5)</td>
<td>240 (49.7)</td>
<td>0.0009</td>
<td>1.69 (1.24-2.31)</td>
</tr>
<tr>
<td><em>KIR2DS5</em> centromeric</td>
<td>38 (15.1)</td>
<td>112 (23.2)</td>
<td>0.0095</td>
<td>0.59 (0.39-0.88)</td>
</tr>
<tr>
<td><em>KIR2DS5</em> telomeric</td>
<td>20 (8)</td>
<td>39 (8.1)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><em>KIR2DS5</em> on a contracted <em>KIR</em> haplotype</td>
<td>22 (8.8)</td>
<td>50 (10.4)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><em>KIR2DS5</em> on other <em>KIR</em> genotypes</td>
<td>14 (5.6)</td>
<td>42 (8.7)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher's exact test with mid-p adjustment
Table S4 Frequency of the different KIR2DS5 alleles

<table>
<thead>
<tr>
<th>KIR2DS5 allele location</th>
<th>KIR2DS5 allele</th>
<th>Pre-eclampsia cases (N=251) n (%)</th>
<th>Controls (N=483) n (%)</th>
<th>P-value*</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cB</td>
<td>*004</td>
<td>22 (8.8)</td>
<td>39 (8.1)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>cB</td>
<td>*00502</td>
<td>2 (0.8)</td>
<td>3 (0.6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>cB</td>
<td>*006</td>
<td>10 (4)</td>
<td>51 (10.6)</td>
<td>0.0015†</td>
<td>0.35 (0.17-0.69)</td>
</tr>
<tr>
<td>cB</td>
<td>*007</td>
<td>13 (5.2)</td>
<td>23 (4.8)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>cB</td>
<td>*010</td>
<td>3 (1.2)</td>
<td>17 (3.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>cB and tB†</td>
<td>*005</td>
<td>26 (10.4)</td>
<td>66 (13.7)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>cB</td>
<td>*005C</td>
<td>14 (5.6)</td>
<td>33 (6.8)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tB</td>
<td>*005T</td>
<td>6 (2.4)</td>
<td>14 (2.9)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>*005 others</td>
<td>6 (2.4)</td>
<td>19 (3.9)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tB</td>
<td>*002</td>
<td>28 (11.2)</td>
<td>55 (11.4)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tB</td>
<td>*003</td>
<td>7 (2.8)</td>
<td>17 (3.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tB</td>
<td>*008</td>
<td>2 (0.8)</td>
<td>2 (0.4)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tB</td>
<td>*009</td>
<td>12 (4.8)</td>
<td>25 (5.2)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tB</td>
<td>*011</td>
<td>1 (0.4)</td>
<td>4 (0.8)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher's exact test with mid-p adjustment
†P=0.0205 after Bonferroni correction
‡KIR2DS5*005 can be found in both the cB and tB region. The more detailed analysis is given when the assignment to each region is possible.
Table S5 Frequency of maternal and fetal HLA-C genotypes

<table>
<thead>
<tr>
<th>HLA-C genotype</th>
<th>Mothers Pre-eclampsia cases (n=251)</th>
<th>Controls (n=483)</th>
<th>P-value</th>
<th>Fetuses Pre-eclampsia cases (n=247)</th>
<th>Controls (n=480)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-C1C1</td>
<td>46 (18.3)</td>
<td>95 (19.7)</td>
<td>NS</td>
<td>45 (18.2)</td>
<td>106 (22.1)</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-C1C2</td>
<td>132 (52.6)</td>
<td>245 (50.7)</td>
<td>NS</td>
<td>118 (47.8)</td>
<td>227 (47.3)</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-C2C2</td>
<td>73 (29.1)</td>
<td>143 (29.6)</td>
<td>NS</td>
<td>84 (34)</td>
<td>147 (30.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**HLA-C group frequency**

<table>
<thead>
<tr>
<th>HLA-C</th>
<th>Mothers Pre-eclampsia cases (n=251)</th>
<th>Controls (n=483)</th>
<th>P-value</th>
<th>Fetuses Pre-eclampsia cases (n=247)</th>
<th>Controls (n=480)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-C1</td>
<td>224 (44.6)</td>
<td>435 (45)</td>
<td>NS</td>
<td>208 (42.1)</td>
<td>439 (45.7)</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-C2</td>
<td>278 (55.4)</td>
<td>531 (55)</td>
<td>NS</td>
<td>286 (57.9)</td>
<td>521 (54.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table S6 Frequency of maternal KIR and fetal HLA-C combinations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-eclampsia cases (n=246)</th>
<th>Controls (n=478)</th>
<th>P-value*</th>
<th>Risk compared to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trend</td>
<td></td>
<td></td>
<td>0.0122</td>
<td></td>
</tr>
<tr>
<td><strong>KIR AA mother</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal HLA-C2C2</td>
<td>29 (11.8)</td>
<td>39 (8.2)</td>
<td>1.847</td>
<td></td>
</tr>
<tr>
<td>Fetal HLA-C1C2</td>
<td>46 (18.7)</td>
<td>67 (14)</td>
<td>1.705</td>
<td></td>
</tr>
<tr>
<td>Fetal HLA-C1C1</td>
<td>14 (5.7)</td>
<td>29 (6.1)</td>
<td>1.199</td>
<td></td>
</tr>
<tr>
<td><strong>KIR AB or BB mother</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal HLA-C2C2</td>
<td>54 (22)</td>
<td>106 (22.2)</td>
<td>1.265</td>
<td></td>
</tr>
<tr>
<td>Fetal HLA-C1C2</td>
<td>72 (29.3)</td>
<td>160 (33.5)</td>
<td>1.118</td>
<td></td>
</tr>
<tr>
<td>Fetal HLA-C1C1</td>
<td>31 (12.6)</td>
<td>77 (16.1)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Extended Mantel-Haenszel chi square for linear trend
Supplementary Figures.

Fig. S1. Birth weight (g) distributions of babies from control (n=484) (dotted line) and pre-eclamptic (solid line) (n=229) pregnancies. The birth weight (g) is shown on the x axis and the frequency (%) on the y axis.

Fig. S2. Linear trends in the frequencies of the maternal KIR and fetal HLA-C genotype combinations depicted in Table S5. Mothers were grouped as having either KIR AA or KIR AB/BB genotypes. Fetal HLA-C genotypes were defined as HLA-C2C2, HLA-C1C2 or HLA-C1C1. In a comparison of pre-eclamptic and control pregnancies, there is a significant linear trend in frequencies. The most risk of pre-eclampsia is in pregnancies with a KIR AA mother and a HLA-C2C2 or HLA-C1C2 fetus. The least risk is with KIR AB/BB mothers with HLA-C1C2 or HLA-C1C1 fetuses. The data was analysed using an extended Mantel-Haenszel test for linear trend (p=0.0122).
Figure S1
**Figure S2**

Extended Mantel-Haenszel chi-square for linear trend

P = 0.0122

**Bar Graph**

- HLA-C2C2 fetus
- HLA-C1C2 fetus
- HLA-C1C1 fetus

**KIR AA mother**

- Controls (N=478)
- Pre-eclampsia cases (N=246)

**KIR AB or BB mother**

- Controls (N=478)
- Pre-eclampsia cases (N=246)