A *KIR B* centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia

Annettee Nakimuli[a,1], Olympe Chazara[b,c,1], Susan E. Hiby[b,c], Lydia Farrell[b,c], Stephen Tukwasibwe[a], Jyothi Jayaraman[d], James A. Traherne[d,e], John Trowsdale[d,e], Francesco Colucci[f,g], Emma Lougee[h], Robert W. Vaughan[h], Alison M. Elliott[i,j], Josaphat Byamugisha[a], Pontiano Kaleebu[i], Florence Mirembe[a], Neda Nemat-Gorgani[k,l], Peter Parham[k,l], Paul J. Norman[k,l], and Ashley Moffett[b,c,2]

[a]Department of Obstetrics and Gynaecology, Makerere University, Kampala, Uganda; [b]Department of Pathology, [c]Centre for Trophoblast Research, and [e]Division of Immunology, Department of Pathology, University of Cambridge, Cambridge, CB2 1QP, United Kingdom; [d]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, CB2 0XY, United Kingdom; [f]Department of Obstetrics and Gynaecology, Centre for Trophoblast Research, University of Cambridge, Cambridge, CB2 OWS, United Kingdom; [g]National Institute for Health Research Cambridge Biomedical Research Centre, University of Cambridge School of Clinical Medicine, Cambridge, CB2 OWS, United Kingdom; [h]Clinical Transplantation Laboratory, Viapath, Guy's Hospital, London, SE1 9RT, United Kingdom; [i]Medical Research Council/Uganda Virus Research Institute Uganda Research Unit on AIDS, Entebbe, Uganda; [j]Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, United Kingdom; and Departments of [k]Structural Biology and [l]Microbiology and Immunology, Stanford University, Stanford, CA 94305-5126

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In sub-Saharan Africans, maternal mortality is unacceptably high, with >400 deaths per 100,000 births compared with <10 deaths per 100,000 births in Europeans. One-third of the deaths are caused by pre-eclampsia, a syndrome arising from defective placentation. Controlling placentation are maternal natural killer (NK) cells that use killer-cell immunoglobulin-like receptor (KIR) to recognize the fetal HLA-C molecules on invading trophoblast. We analyzed genetic polymorphisms of maternal *KIR* and fetal *HLA-C* in 484 normal and 254 pre-eclamptic pregnancies at Mulago Hospital, Kampala, Uganda. The combination of maternal *KIR AA* genotypes and fetal *HLA-C* alleles encoding the C2 epitope associates with pre-eclampsia [$P = 0.0318$, odds ratio (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.

Uganda | pre-eclampsia | NK cells | maternal mortality | KIR

Although pre-eclampsia presents clinically with a diverse array of systemic symptoms, the underlying disease-causing mechanism starts with placentation when trophoblast cells invade the decidua. Here, they transform the uterine spiral arteries into large vessels that form the fetoplacental supply line (1, 2). In pre-eclampsia and other pregnancy disorders (fetal growth restriction, stillbirth, and recurrent miscarriage) known collectively as the great obstetric syndromes (GOSs), trophoblast fails to invade optimally (3). Pre-eclampsia and other GOSs occur in all populations, but women of African ancestry are significantly more at risk; thus, GOSs are responsible for much of the high maternal and fetal mortality rates seen in sub-Saharan Africa (SSA) (4). The genetic contribution to pre-eclampsia is supported by several studies and involves both maternal genes and paternal genes inherited by the fetus (5, 6).

The wall of the uterus is the territorial boundary between two genetically different individuals: the mother and the fetus. The uterine mucosal immune system appears to define this maternal/placental boundary. The decidua must control placentation, because in its absence, the trophoblast infiltrates to a dangerous extent, causing the condition of placenta percreta (7). The decidua contains an abundant population of specialized natural killer (NK) cells. These uterine NK cells (uNK) express killer-cell immunoglobulin-like receptors (KIRs) that recognize trophoblast HLA-C ligands (8, 9). Both *KIR* and *HLA-C* are genetically variable, resulting in many possible combinations of maternal *KIR* and fetal HLA-C ligands (10). The *KIR* region is defined by two groups of haplotype: *A* and *B*. The *KIR A* haplotype has seven *KIR* genes, all encoding inhibitory receptors apart from *KIR2DS4*. In contrast, the *KIR B* haplotype contains a variable number of additional *KIR*, most of which encode activating receptors (11, 12). All HLA-C allotypes are KIR ligands and can be divided into two groups (carrying either C1 or C2 epitopes) that are distinguished by a dimorphism at position 80 and recognized by different KIR (13). Within a human population, the combination of *KIR* and *HLA* diversity distinguishes individuals and this extremely high variation is particularly evident in SSA

Significance

Pre-eclampsia is especially common in women of African ancestry and a major cause of maternal death. The killer-cell immunoglobulin-like receptor (*KIR*) genes that we analyzed are expressed 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. 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.

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[1]A.N. and O.C. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: am485@cam.ac.uk.

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populations. They exhibit less linkage disequilibrium (LD) between the *KIR* genes than other populations (14–16), and the *KIR* genes have greater allelic diversity (15, 16). A variety of diseases and clinical conditions has been associated with combinations of *HLA-C* and *KIR* genes. In previous case–control studies of pre-eclampsia in pregnant European women, we showed that, when the fetus carries a C2 epitope, maternal *KIR AA* genotypes are risk factors for pre-eclampsia, whereas the *KIR2DS1* gene of maternal *KIR B* haplotypes is protective (8, 17). In the case–control study reported here, we test the hypothesis that these factors confer similar risk and protection to pregnant SSA women.

Results

Clinical Characteristics of the Cohort. This case–control study of pre-eclampsia involved 738 pregnant women at Mulago Hospital in Kampala, 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 of the groups were similar (Table S1). HIV+ women were not excluded from the analysis, because there were similar numbers in both pre-eclamptic and control pregnancies (~5%) (Table S1), and similar results were found, even when HIV+ women were omitted (Table S2). As expected, gestational age at delivery and birth weight were significantly lower in the pre-eclamptic cases compared with controls ($P < 0.001$) (Fig. S1 and Table S1).

Unlike European Women, *KIR B* Centromeric Regions Containing *KIR2DS5* Protect Ugandan Women from Pre-eclampsia. Maternal *KIR AA* genotype is increased in pre-eclamptic pregnancies [$P = 0.0256$, odds ratio (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) (Fig. 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 genes, only *KIR2DS5* is significantly protective for women with pre-eclampsia after Bonferroni correction ($P = 0.0009$, $P_c = 0.0126$, OR = 0.59) (Fig. 2*A*, Table 1, and Table S3). In comparable studies on European women, protection was seen with *KIR2DS1* and not seen with *KIR2DS5*, which is shown here for African women (Table 1). Moreover, in Ugandans, the telomeric *B* (*tB*) genes *KIR2DS1* and *KIR3DS1* are at similar low frequency in cases and controls (Table 1).

Because *KIR* genes are in LD, *KIR2DS5* could be itself protective or marking a nearby protective gene. *KIR2DS5* can be found in both the *KIR* centromeric *B* (*cB*) and *tB* 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 (*Materials and Methods* and Fig. 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 10 alleles that were assigned to *cB* or *tB* regions as described in *Materials and Methods* (Fig. 4). *KIR2DS5*004*, *KIR2DS5*006*, *KIR2DS5*007*, and *KIR2DS5*010* are restricted to *cB*, whereas *KIR2S5*002*, *KIR2S5*003*, *KIR2S5*008*, *KIR2S5*009*, and *KIR2S5*011* are restricted to *tB*. *KIR2DS5*005* is the most frequent allele and the only one found in both *cB* and *tB* (Fig. 4), pointing to it being the progenitor of all other *KIR2DS5* alleles. Our assignments of *KIR2DS5* alleles to *cB* or *tB* agree with those defined by complete *KIR* haplotype sequences and analyses 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

Table 1. Frequency of maternal *KIR* genotypes and *KIR* gene carriers

KIR genotypes or individual *KIR* genes present	Uganda pre-eclampsia cases ($n = 251$) *n* (%)	Uganda controls ($n = 483$) *n* (%)	*P* value*	OR (95% CI)	United Kingdom pre-eclampsia cases ($n = 729$) *n* (%)†	United Kingdom controls ($n = 592$) *n* (%)†	*P* value*	OR (95% CI)
KIR genotype								
KIR AA	91 (36.3)	136 (28.2)	0.0256	1.45 (1.05–2.01)	266 (36.5)	163 (27.5)	0.0005	1.51 (1.20–1.91)
KIR AB	157 (62.5)	336 (69.6)	NS		456 (62.6)	424 (71.6)		
KIR BB	3 (1.20)	11 (2.28)	NS		7 (0.96)	5 (0.84)		
KIR genes								
2DP1	247 (98.4)	474 (98.1)	NS		NA	NA		
2DL1	247 (98.4)	476 (98.6)	NS		707 (97.0)	569 (96.1)	NS	
2DL2	132 (52.6)	293 (60.7)	0.0365	0.72 (0.53–0.98)	348 (47.7)	313 (52.9)	NS	
2DL3	222 (88.4)	414 (85.7)	NS		662 (90.8)	530 (89.5)	NS	
2DL5	138 (55.0)	316 (65.4)	0.0061	0.65 (0.47–0.88)	330 (45.3)	330 (55.7)	0.0002	0.66 (0.53–0.82)
3DL1	248 (98.8)	473 (97.9)	NS		600 (95.5)‡	517 (94.3)‡	NS	
3DS1	30 (12.0)	57 (11.8)	NS		211 (33.8)‡	242 (44.3)‡	0.0002	0.64 (0.51–0.81)
2DS1	52 (20.7)	114 (23.6)	NS		240 (32.9)	255 (43.1)	0.0002	0.65 (0.52–0.81)
2DS2	118 (47.0)	262 (54.2)	NS		349 (47.9)	317 (53.5)	NS	
2DS3	56 (22.3)	118 (24.4)	NS		185 (25.4)	175 (29.6)	NS	
2DS4	244 (97.2)	462 (95.7)	NS		703 (96.4)	560 (94.6)	NS	
2DS4 del	73 (29.1)	144 (29.9)	NS		632 (89.9)	474 (84.8)	NS	
2DS4 wt	171 (68.1)	318 (65.8)	NS		262 (37.3)	215 (38.5)	NS	
2DS5	94 (37.5)	243 (50.3)	0.0009§	0.59 (0.43–0.81)	205 (28.1)	214 (36.1)	0.0023	0.69 (0.55–0.87)

CI, confidence interval; NA, not available; NS, not significant.
*Fisher's exact test with mid-p adjustment.
†Ref. 8.
‡A number of individuals were not typed for this gene.
§$P = 0.0126$ after Bonferroni correction.

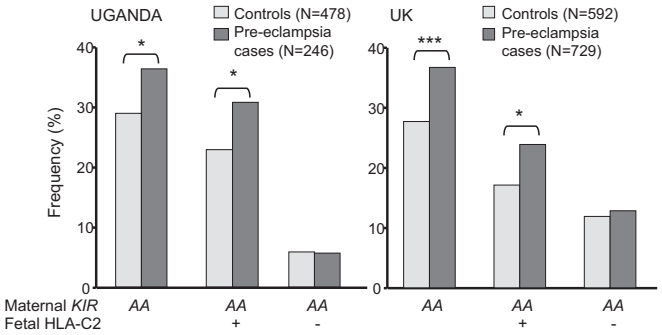

Fig. 1. Frequency of the *KIR AA* genotype alone and combined with the fetal *HLA-C* carrier group in Uganda and the United Kingdom. There was a significant difference in the *KIR AA* genotype frequencies between controls (light gray bars) and pre-eclampsia cases (dark gray bars) in both the Ugandan (**P* = 0.0256, OR = 1.45) and the United Kingdom (****P* = 0.0005, OR = 1.51) cohorts. The frequency of *KIR AA* genotypes is shown combined with either fetuses carrying a C2 epitope or those lacking C2 and carrying only C1-bearing HLA-C allotypes. There is a significant risk of pre-eclampsia when a *KIR AA* woman has a fetus carrying a C2 epitope for both cohorts. In Uganda, **P* = 0.0318, and OR = 1.49, and in the United Kingdom, **P* = 0.0267, and OR = 1.46.

the protection that they provide against pre-eclampsia. *KIR2DS5* is protective in Ugandan women when it is present in the *cB* region (*cB01* or *cB03*; $P = 0.0095$, OR = 0.59) (Figs. 2*B* and 3 and Table S3). Furthermore, of all of the *cB KIR2DS5* alleles, only *KIR2DS5*006* is significantly more frequent in controls than in pre-eclamptic pregnancies ($P = 0.0015$, OR = 0.35) (Fig. 2*C* and 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, seems neutral. Consistent with the low frequency of *KIR2DS1* and *KIR3DS1* in Africans, *KIR2DS5* is less frequently present in *tB* than *cB*. When present in *tB*, it has no effect, being at similar frequencies in controls and cases (Fig. 2*B* and 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* (Fig. 3).

In Ugandan Women, Like European Women, Pre-eclampsia Associates with Maternal *KIR AA* 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 allotypes. 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 the greatest risk ($P = 0.0122$) (Fig. S2 and 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 (not significant; OR = 1.16, 95% confidence interval = 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 *KIR2DS5* when the fetus has an *HLA-C* allele encoding a C2 epitope inherited from its father.

Recurrence of Pre-eclampsia in Ugandan Women Is Associated with Maternal *KIR AA* Genotype and Fetal Expression of Paternal *HLA*-C2. The risk of recurrence of pre-eclampsia is known to be high (~20%) (20, 21). In our cohort, there 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 eleven *KIR AA* pregnancies in this subcohort 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 for 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, because unexplained stillbirth, fetal growth restriction, and preterm labor are more common in 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 the *KIR* locus in Africans both at the level of *KIR* haplotypes and in the number of alleles at individual *KIR* genes (10, 15, 16). Despite this complexity, we find complete consistency with our studies of

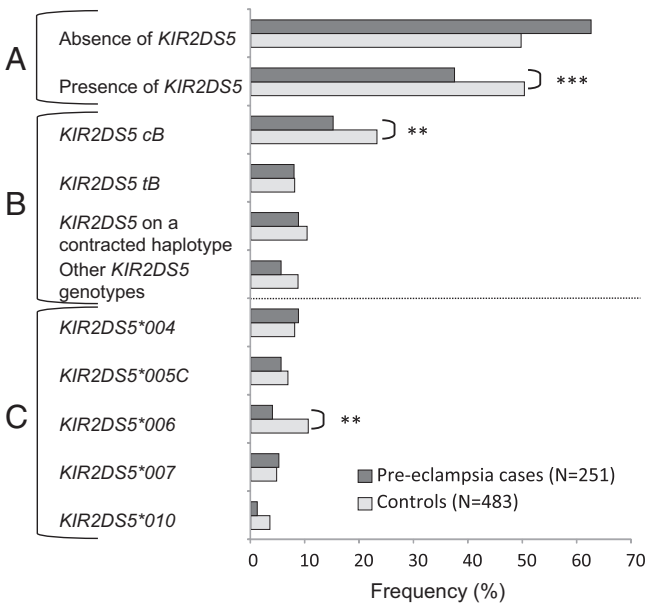

Fig. 2. Frequencies of the different genotypes carrying *KIR2DS5* in controls and pre-eclampsia cases. (*A*) All controls (light gray bars) and pre-eclamptic cases (dark gray bars) were grouped according to whether they carried *KIR2DS5*. The presence of *KIR2DS5* protects women from pre-eclampsia. ****P* = 0.0009, OR = 0.59 ($P_c = 0.0126$ after Bonferroni correction). (*B*) Women were grouped according to the location of *KIR2DS5* on the *KIR B* haplotype (*cB*, *tB*, contracted, or other unusual genotypes). *KIR2DS5* on *cB* is significantly protective. ***P* = 0.0095, OR = 0.59. (*C*) The carrier frequencies of those *KIR2DS5* alleles present on *cB* were compared between controls and pre-eclamptic cases. *KIR2DS5*005C* indicates those women in whom *KIR2DS5* is located on *cB*. Only *KIR2DS5*006* is significantly protective. ***P* = 0.0015, OR = 0.35.

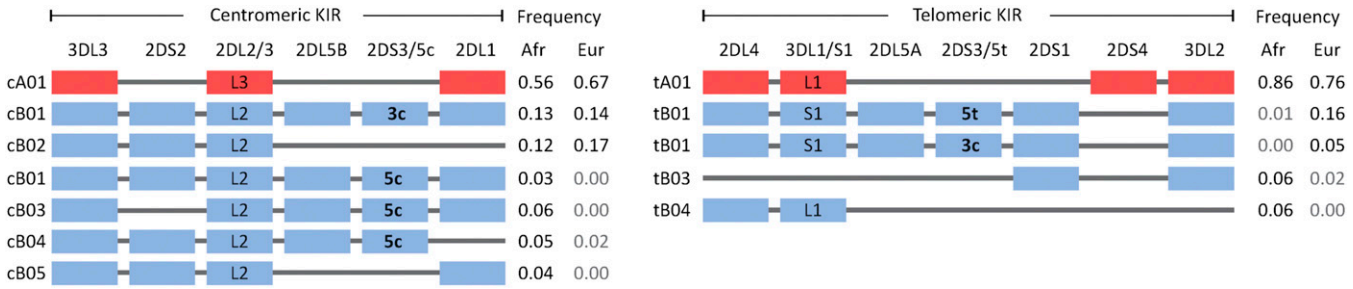

Fig. 3. Component genes of centromeric (c) and telomeric (t) *KIR* haplotype segments in African and European populations. The red segments together form the *KIR A* haplotype, and all other combinations of centromeric and telomeric motifs form *KIR B* haplotypes. The gene content motifs, shown for the (*Left*) centromeric and (*Right*) telomeric regions, are named according to (19), where *KIR2DS3* and *KIR2DS5* were considered as alleles of two genes, centomeric *KIR2DS3/5* and telomeric *KIR2DS3/5*. The frequencies of the different *KIR* regions in representative African and European populations are also shown (15, 39). Afr, African; Eur, European.

pre-eclampsia in Europeans: the risk is associated with a maternal *KIR AA* genotype combined with a paternally derived HLA-C allotype 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 AA* genotypes in the women in our study is striking (45.8% compared with 28.2% in controls) (21). The gene always present on the *KIR A* haplotype likely to confer this risk is *KIR2DL1*, which encodes for an inhibitory KIR with strict specificity for C2 epitopes (22). Thus, in women with a *KIR AA* genotype containing two copies of *KIR2DL1*, uNK will be strongly inhibited when confronted by trophoblast expressing C2-bearing HLA-C. There are at least 12 *KIR2DL1* alleles located in the centromeric *A* (*cA*) region in Africans compared with 1–5 in other populations (15). 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 with 64% for European women. Similarly, the probability of African women having a fetus carrying a paternal C2 epitope is 55% compared with 40% for European women (Table S5). 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 Hepatitis C and HIV (10, 23–25). In SSA, possession of 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.

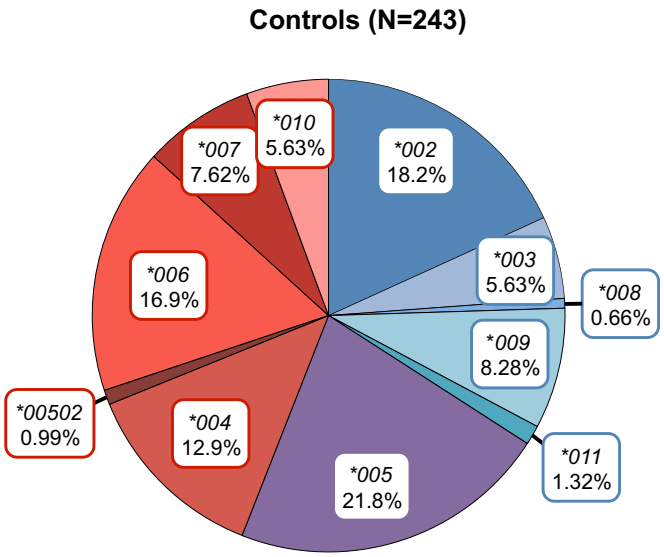

Fig. 4. Carrier frequencies of the different *KIR2DS5* alleles found in the Ugandan population; *cB* alleles are in shades of red, *tB* alleles are in shades of blue, and *KIR2DS5*005* (purple) is found in both *cB* and *tB*.

We observed that *tB* 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 with 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 *KIR2DS5* allele in Europeans, *KIR2DS5*002*, is in tight LD with *KIR2DS1* and located in the *tB* region. Unlike Europeans, however, *KIR2DS5* is polymorphic in Africans and African Americans. We found 10 alleles in Ugandans, consistent with previous reports from African Americans [the Immuno Polymorphism Database (IPD)], located in both *cB* and *tB* but most commonly found in those *cB* regions that also contain *KIR2DP1* and *KIR2DL1* (26, 27). The dominant allele, *KIR2DS5*005*, is the only allele found in both *cB* and *tB*, and it is probably ancestral; when in either location, it was similar in frequency between cases and controls. Of *cB KIR2DS5* alleles, only *KIR2DS5*006* is significantly associated with protection from pre-eclampsia. KIR2DS5 can be expressed by European peripheral blood NK cells, but we have been unable to show its expression on uNK using similar reagents (28–30). The functional effects of KIR2DS5 diversity await additional investigation, but certain KIR2DS5 allotypes do show different expression levels in transfected cells, similar to findings for other KIR variants (30). For example, allelic variation of *KIR2DL1* affects protein expression levels at the cell surface, NK repertoire, and affinity of binding (22, 31, 32). Furthermore, although no binding has been shown of the European allele *KIR2DS5*002* to any HLA ligand, *KIR2DS5*006* might bind to C2-bearing HLA-C allotypes common in Africans (C*04, C*02, C*17, and C*18) (15).

Another possibility is that *KIR2DS5* is in LD with other *KIR* on the protective *cB01* and *cB03* regions, notably *KIR2DL1*. The *cB KIR2DL1* allele present in Europeans, *KIR2DL1*004*, gives a weak inhibitory signal compared with the common *cA* allele, *KIR2DL1*003* (31). Thus, the protective effect of the *cB01* and

Table 2. Risk of pre-eclampsia associated with the absence of *KIR2DS5* for the different maternal/fetal HLA-C combinations

Parameter	*P* value*	OR (95% confidence interval)
Effect of relative dose of maternal and fetal HLA-C2		
Fetus had fewer C2 than the mother	0.7085	1.09 (0.69–1.69)
Fetus had the same number of C2 as the mother	0.1612	1.28 (0.91–1.80)
Fetus had more C2 than the mother	0.0130†	1.72 (1.12–2.64)
Effect of origin of fetal HLA-C2		
Paternal origin	0.0203†	1.80 (1.10–2.93)
Maternal origin	0.5222	1.16 (0.72–1.84)

*Fisher's exact test with mid-p adjustment.
†$P < 0.05$.

cB03 regions might be due to either KIR2DS5 activation or weaker KIR2DL1 inhibition, because both could counterbalance the strong inhibition conferred from *cA KIR2DL1* alleles. For both *KIR A* and *B* haplotypes, the particular *KIR2DS5* and *KIR2DL1* alleles involved are, therefore, important, but to investigate this will require much larger, clinically well-characterized cohorts. Our method to infer *KIR* regions allows a fairly simple analysis of *KIR* data from clinical cohorts in SSA compared with the complex sequencing needed to define the exact haplotypes (15). Hence, although this analysis does not unravel the complete complexity of *KIR* variants found, it can point to the regions conferring risk or protection. In this clinical context, we have a clear pointer that the *cB01* and *cB03* regions containing *KIR2DS5*, *KIR2DL1*, and *KIR2DP1* are providing protection from pre-eclampsia in Ugandan women.

In this African cohort, such as in Europeans, a paternal rather than a maternal origin of fetal C2 confers risk in women lacking *KIR2DS5* (8). Whether this effect is caused by disparities between individual maternal and paternal HLA-C2 allotypes (allogeneic) and/or a dosage effect (more *HLA-C* alleles encoding C2 in the fetus than in the mother when C2 is paternally derived) is unresolved (8). To understand this effect will require high-resolution genotyping of *C1C2* mothers who have *C1C2* babies (where the dosage is identical) in a large cohort (2,000 cases and 4,000 controls would be required).

The great diversity of *KIR* and *HLA-C* variants in SSA is maintained by balancing selection (16). The two contrasting functions of these immune system gene families in reproduction and immune responses to infection mean that certain variants will be important at different stages of life in women, men, children, and adults and in geographical regions with a range of different pathogens. We have previously argued that the selective pressures from reproductive success and immune response to pathogens are competing and have driven evolution of the *KIR A* and *B* haplotypes in humans compared with other hominids (10). Our combined studies of *KIR*/*HLA-C* variants in diverse European and African populations now suggest that the unusual reproductive strategies characteristic of modern humans compared with other hominids could also be a cause of balancing selection. The evolution of the large neonatal brain relative to a pelvis adapted for bipedalism means that birth weight must be kept between two strictly defined limits. When babies are large (>95th centile), there is a risk of cephalopelvic disproportion and subsequent prolonged obstructed labor, birth asphyxia, and postpartum hemorrhage. Furthermore, these outcomes, like pre-eclampsia and other GOS, are also much more common in African women with associated features of pregnancy that favor smaller babies: earlier birth (the gestational age is reduced to 38 wk), the head engages late into the pelvis, and the baby matures earlier than in non-Africans (4). Thus, there is high mortality in mother and babies not only 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 allows 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 cephalopelvic disproportion (4), there is even greater selection for reduced fetal size with associated pre-eclampsia; this effect 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 extremes, but in pre-eclampsia and low birth weight (less than fifth centile), the risk is with maternal *KIR AA* genotypes, whereas in high birth weight, the association is with maternal *KIR2DS1* (33). Studies on how these genetic findings are translated in uNK functional differences are still limited, but we found that, when KIR2DS1+ uNKs (isolated from United Kingdom patients) are activated by target cells expressing HLA-C2, there is increased production of soluble factors [e.g., granulocyte-macrophage colony-stimulating factor (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. *tB* regions and *KIR2DS1* are infrequent in Africans compared with Europeans, but the opposite is true for *cB* regions containing *KIR2DS5*. During the out-of Africa migrations, it is possible that only individuals having *tB* with *KIR2DS1* moved away from SSA. Introgression of *KIR2DS1* from archaic humans is also a possibility (35). Our previous findings do indicate that *KIR2DS1* and *KIR3DS1* (both on *tB*) are selected against in SSA (14, 16). Studying disorders of pregnancy in an African setting is important and informative; the high rates of pre-eclampsia as well as other major disorders of pregnancy, including obstructed labor and stillbirth, and the greater genetic diversity of *KIR* in SSA mean that unraveling the role of the complex *KIR* and *HLA* systems will provide valuable genetic information to predict women who are at risk for a range of pregnancy disorders.

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. The participants gave written informed consent to participate in the study. Withdrawal from the study never jeopardized healthcare, which 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 SSA, with over 30,000 deliveries a year. Genomic DNA was obtained from maternal blood from unrelated healthy women ($n = 484$) and women with pre-eclampsia or eclampsia ($n = 254$) between July of 2009 and June of 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 h apart plus proteinuria of +2 or more by dipstick both at 20 wk or more of gestation. Eclampsia was diagnosed when a patient with pre-eclampsia had generalized tonic–clonic convulsions. Controls were women with a normal first pregnancy delivering at term (≥38 wk) 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 were collected at the time of clinical examination of the participants using an interviewer-administered questionnaire, and additional information was obtained from medical charts.

DNA Isolation and Genotyping. Maternal genomic DNA was isolated from 5 mL blood using the QIAamp DNA Maxi Blood Kit (Qiagen). Fetal DNA was isolated from umbilical cord samples after overnight incubation with Proteinase K (Roche) and purification with a protein precipitation solution (Qiagen) followed by ethanol precipitation. Twelve maternal *KIR* genes were typed for presence or absence by PCR with sequence specific primers (PCR-SSP) using two pairs of primers per gene or allele as described previously (8, 14, 36). The *KIR* genes typed were *2DL1*, *2DL2/3*, *2DL5*, *3DL1/S1*, *2DP1*, *2DS1*, *2DS2*, *2DS3*, *2DS4* (including the deletion), and *2DS5*. All of the samples were typed for *KIR2DL1* and *KIR2DP1* copy number, and 28 selected samples were further investigated for additional *KIR* (*2DL4*, *3DP1*, *3DL2*, and *3DL3)* so that all 14 *KIR* genes were included (37). Individual genotypes were defined according to their combination of centromeric (*cA* and *cB*) and telomeric (*tA* and *tB*) *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*, *2DL2/3*, *2DP1*, *2DL1*, *3DL1/S1*, *2DS1*, and *2DS4*. There are common *cB* regions in Africans (Fig. 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 *2DP1*, *2DL1*, *2DL5*, and *2DS5* (or *2DS3*), whereas *cB02* lacks these genes. *KIR2DS5* alleles were genotyped by pyrosequencing; targeting exons 5–7 (15). Then, by knowing which *KIR2DS5* alleles are present in individuals homozygous for either *cA* or *tA* regions, we could assign each of 10 *KIR2DS5* alleles to *cB* or *tB* (Fig. 4). C1 and C2 were defined in maternal and fetal samples based on the primers and methods described previously (8, 36). *HLA-C* low-resolution allelic typing was performed using a PCR-SSP method consisting of 21 reaction wells that was adapted from ref. 38. Each well contained a final reaction volume of 10 μL consisting of 5× Green GoTaq Flexi Buffer (Promega), 0.2 mM dNTPs (ThermoFisher), 1.25 mM MgCl2 (Promega), 0.4 U GoTaq DNA polymerase (Promega), 134 nM 63/64 control primer (Eurogentec), and ∼45 ng 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 were analyzed using the χ^2 and Fisher's exact tests with two-tailed mid-p adjustment and Student's *t* tests for continuous data. A *P* value of $\leq$0.05 was considered to be statistically significant. The magnitude of the effect was estimated by ORs and their 95% confidence intervals.

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