Diversity of KIR genes and their HLA-C ligands in Ugandan populations with historically varied malaria transmission intensity

Stephen Tukwasibwe,1,2 James A. Traherne,3 Olympe Chazara,3,4 Jyothi Jayaraman,3,4 John Trowsdale,3 Ashley Moffett,3,4 Wei Jiang,3 Joaniter I. Nankabirwa,1,2 John Rek,2 Emmanuel Arinaitwe,2 Samuel L. Nsobya,1,2 Maxine Atuheirwe,1 Mubiru Frank,1 Anguzi Godwin,1 Prasanna Jagannathan,5 Stephen Cose,6 Moses R. Kamya,1,2 Grant Dorsey,7 Philip J. Rosenthal,7 Francesco Colucci,4,8 and Annettee Nakimuli1*

1Makerere University College of Health Sciences, Kampala, Uganda; 2Infectious Diseases Research Collaboration; 3University of Cambridge, Department of Pathology, Cambridge, United Kingdom; 4University of Cambridge Centre for Trophoblast Research; 5Stanford University, School of Medicine, USA; 6MRC/UVRI and LSHTM Uganda Research Unit, Kampala, Uganda; 7University of California, San Francisco, USA; 8Department of Obstetrics & Gynaecology, University of Cambridge, National Institute for Health Research Cambridge Biomedical Research Centre, Cambridge CB2 0SW, UK.

E-mail addresses of authors: stephentukwasibwe@yahoo.com, jat51@cam.ac.uk, olympechazara@gmail.com, jj329@cam.ac.uk, jt233@cam.ac.uk, am485@cam.ac.uk, wj224@cam.ac.uk, jnankabirwa@idrc-uganda.org, jrek@idrc-uganda.org, earinaitwe@idrc-uganda.org, samnsobya@yahoo.co.uk, maxinejasmine194@gmail.com, mubiruf09@gmail.com, tukumbgodwin@gmail.com, prasj@stanford.edu, stephen.cose@lshtm.ac.uk, mkamya@infocom.co.ug, grant.dorsey@ucsf.edu, philip.rosenthal@ucsf.edu, fc287@medschl.cam.ac.uk, annettee.nakimuli@gmail.com

Key words: Genetic diversity, Human Leukocyte Antigen, Killer-cell immunoglobulin-like receptor, malaria, Uganda
Abstract

Background: Malaria is one of the most serious infectious diseases in the world. The malaria burden is greatly affected by human immunity, and immune responses vary between populations. Genetic diversity in KIR and HLA-C genes, which are important in immunity to infectious diseases, is likely to play a role in this heterogeneity. Several studies have shown that KIR and HLA-C genes influence the immune response to viral infections, but few studies have examined the role of KIR and HLA-C in malaria infection, and these have used low-resolution genotyping. Our aim was to determine whether genetic variation in KIR and their HLA-C ligands differ in Ugandan populations with historically varied malaria transmission intensity using more comprehensive genotyping approaches.

Methods: We used high throughput multiplex quantitative real-time PCR method to genotype KIR genetic variants and copy number variation and developed a high-throughput real-time PCR method to genotype HLA-C1 and C2 allotypes for 1,344 participants, aged 6 months to 10 years, enrolled from Ugandan populations with historically high (Tororo District), medium (Jinja District) and low (Kanungu District) malaria transmission intensity.

Results: The prevalence of KIR3DS1, KIR2DL5, KIR2DS5 and KIR2DS1 genes was significantly lower in populations from Kanungu compared to Tororo (7.6% vs. 13.2%: p=0.006, 57.2% vs. 66.4%: p=0.005, 33.2% vs. 46.6%: p<0.001 and 19.7% vs. 26.7%: p=0.014 respectively) or Jinja (7.6% vs.18.1%: p<0.001, 57.2% vs. 63.8%: p=0.048, 33.2% vs. 43.5%: p=0.002 and 19.7% vs. 30.4%: p<0.001 respectively). The prevalence of homozygous HLA-C2 was significantly higher in populations from Kanungu (31.6%)
compared to Jinja (21.4%), p=0.043, with no significant difference between Kanungu and Tororo (26.7%), p=0.296.

**Conclusions:** The *KIR3DS1, KIR2DL5, KIR2DS5* and *KIR2DS1* genes are potentially beneficial in malaria as these genes have been positively selected for in places with historically high malaria transmission intensity. The high-throughput multiplex real-time *HLA-C* genotyping PCR method we have developed will be useful in disease association studies involving large cohorts.

**Background**

Malaria is estimated to cause nearly half a million deaths each year worldwide (1). Malaria is a known evolutionary driving force in the selection of several human genetic polymorphisms that protect against malaria. Red blood cell alterations are the most studied genetic abnormalities that impact on malaria (2). These include mutations in the alpha- and beta-globin genes that lead to sickle cell anaemia or thalassemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency and the Duffy antigen protein (3). It has been suggested that many of these polymorphisms were selected in human populations due to their role in protection from the detrimental effects of *P. falciparum* infection (4). It has been demonstrated that different populations have developed independent evolutionary responses to malaria (5). For example, three haemoglobin variants (HbS, HbC, and HbE) appear to confer protection against malaria in different parts of the world (6). The HbS allele is common in Africa, but rare in Southeast Asia, and the opposite is true for the HbE allele (7, 8).

A recent genome wide association study of 17,000 individuals in Africa reported that known genetic variants account for only 11% of the total genetic influence of malaria on the human genome (9). Among other genes potentially influencing malaria responses are those mediating innate immunity, which is important in protection from *P. falciparum* infection. Natural killer (NK) cells play an important role in the innate immune response to malaria infection (10, 11). NK cells are the first cells in peripheral blood to produce interferon gamma.
(IFN-γ) in response to *P. falciparum* infection (11), and they have also been shown to participate in adaptive immunity. Recent evidence indicates a role for NK cells in malaria infection in humans and in mouse models (10, 12). It has been shown that copy number variation (CNV) in *KIR* genes influences immunity to infections (13) and plays an important role in NK cell education (14) through interactions with their *HLA* class I ligands. Hence, the expression of multiple copies of *KIR* genes could potentially lead to enhanced NK cell education, thereby strengthening immunity to pathogens. This has been well studied in viral infections, but not in malaria.

Some studies have demonstrated that individuals may vary in their ability to elicit an innate immune response to malaria infection, with clear implications for disease manifestations (15). Heterogeneity in response could arise from variations in *KIR* and their major ligands, *HLA*-C molecules, that have a direct impact on NK cell functions (11, 16). The frequencies of different *KIR* and *HLA*-C genes vary remarkably across world populations, which might reflect differential selection pressures as well as persistence of ancestral genotypes (17). The *KIR* and *HLA* loci have been suggested to be fast evolving and under positive selection, with pathogen pressure as the driving force (18, 19). Genetic variation of *KIR* and their *HLA*-C ligands across the African continent is not well documented. Several studies have linked high *KIR* and *HLA* genetic diversity in Africa to malaria pressure (20-22). However, data regarding associations between *KIR* and *HLA* variants and malaria have been inconsistent. Since interactions between the genetically diverse *KIR* and *HLA* molecules modulate the functionality of the NK cell response to malaria infections, a better understanding of associations between these genes and malaria risk will be important in understanding the role of immune system genes in malaria pathogenesis.

To date, limited data on the association of *KIR* and their *HLA*-C ligands and malaria risk are available. The few studies that have been carried out have been case-control comparisons of severe versus uncomplicated malaria, with limited genetic information about *KIR* and *HLA* genes. As an alternative approach, to shed light on potential associations between *KIR* and *HLA* genotypes and malaria risk, we have used more comprehensive
genotyping techniques to evaluate the diversity of KIR and their HLA-C ligands in humans living in regions with varied malaria transmission intensity.

Methods

Study samples and populations

We utilized samples from cohorts enrolled at 3 sites in Uganda, Nagongera Sub-county in Tororo District, a rural area in south-eastern Uganda with historically high malaria transmission intensity; Walukuba Sub-county in Jinja District, a peri-urban area near the city of Jinja in south-central Uganda with historically moderate malaria transmission intensity; and Kiihi Sub-county in Kanungu District, a rural area in south-western Uganda with historically low malaria transmission intensity. To establish these cohorts, all households within the 3 sites were enumerated and mapped, and randomly selected households that included at least one resident 6 months to 10 years of age were enrolled, as previously described (23). All the participants enrolled in these cohorts provided thick blood smears and a blood sample for genetic analysis. For this study, all participants whose parents consented to future use of their samples were considered. No a priori power calculation was performed.

Sample collection and DNA purification

Blood samples were collected into EDTA tubes, and DNA was purified from buffy coats using QIAamp DNA Mini Kits (Qiagen), following manufacturer’s instructions with minor modifications. For each sample 300 μl of buffy coat was mixed with 20 μl of kit protease enzyme solution and then 200 μl of lysis buffer, the mixture was vortexed for 15 seconds and incubated at 56°C for 10 minutes, and then 200 μl of absolute ethanol was added. The mixture was vortexed briefly and transferred to a QIAamp column, and the column was spun for 1 minute at 8000 rpm. The column was then washed twice with kit wash buffer, and DNA was eluted by incubating with 80 μl of kit elution buffer at room temperature for 5 minutes followed by centrifugation at 8,000 rpm for 5 minutes. The DNA concentration was
determined using a Qubit fluorimeter (Life Technologies, Carlsbad, CA), and the isolated DNA was stored at −20°C.

**Preparation of DNA for multiplex qPCR**

To prepare genomic DNA for *KIR* and *HLA-C* genotyping as well as *KIR* copy number identification, 10 ng samples of genomic DNA (2.5 µl of 4 ng/µl) were aliquoted into 384-well plates using the Hydra 96 micro dispenser (Art Robbins, San Jose, CA). The DNA was air dried in the plates for subsequent multiplex quantitative PCR assays. Molecular grade water was used in all reactions.

**KIR genotyping by high throughput multiplex real-time qPCR**

Two pairs of primers were used for each gene, as previously described (24). Additional *KIR* primers were designed using sequence information from the immuno polymorphism database-*KIR* (IPD-*KIR*) database (release 2.4.0) to detect rare alleles of *KIR2DS5* and *KIR2DL3* (*KIR2DS5, 2DS5rev2*: TCC AGA GGG TCA CTG GGA and *KIR2DL3, 2DL3rev3*: AGA CTC TTG GTC CAT TAC CG) (25). Samples were genotyped for copy number by multiplexed quantitative PCR for all the *KIR* genes (*KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DP1, 3DP1, 3DL1, 3DL2, 3DL3, and 3DS1) (26). Reactions were carried out in quadruplicate to ensure accuracy of the copy number scoring. Two controls with known copy number and one non-template control were included in each run. Two assays for both *3DL1* and *3DL2* genes that target different exons of the genes were included to identify known fusion genes (27), which are carried on a truncated haplotype (with *2DS4* completely deleted) seen in individuals of African descent. There is a drop in copy number for exon 9 of *3DL1* and exon 4 of *3DL2* (i.e. discordance between the exon 4 and exon 9 copy numbers in the same gene) when the fusion gene is present.

Assays for *2DS4* variants, *2DS4DEL* (a 22-bp deletion in exon 5 that causes a frameshift mutation) and *2DS4WT* (full-length gene) were also included.

**HLA-C genotyping by high throughput multiplex real-time quantitative PCR**

We developed a high throughput real-time qPCR for genotyping *HLA-C* allotypes. For every reaction, we added KAPA SYBR buffer (5µl), forward primer (1µl), reverse primer (1µl) and
water (4µl) to dried DNA in the 384 well plates. *HLA-C1* PCR conditions were: denaturation at 95°C for 3 minutes, 5 cycles of 95°C for 3 seconds and 72°C for 30 seconds, followed by 35 cycles of 95°C for 3 seconds and 70°C for 30 seconds, dissociation at 60°C for 1 second, and finally 95°C. *HLA-C2* PCR conditions were: denaturation at 95°C for 3 minutes, 5 cycles of 95°C for 3 seconds and 72°C for 45 seconds, followed by 40 cycles of 95°C for 3 seconds and 70°C for 45 seconds, dissociation at 60°C for 1 second, and finally 95°C. In each *HLA-C* allotype, primers were used at 5µM concentrations. Primer combinations for C1 and C2 were: C1 = C1Fa and C1Fb with C1R, and C2 = C2F with C2R respectively (Table 1). This method was validated against a large range of samples, the HLA Reference Panel from Coriell with known *HLA-C* allotypes and in families. The sequences for each of the primers used are shown in Table 1.

**KIR and HLA-C genotypes analysis**

*KIR* genotypes were defined following the recommendations from the 2011 *KIR* workshop that was held at Tammsvik, Stockholm, Sweden (28). Briefly, the centromeric A region (*cenA*) was defined by the presence of *KIR2DL3* and *KIR2DL1* and absence of any A haplotype gene, the centromeric B (*cenB*) region was defined by presence of any centromeric B haplotype gene (*KIR2DS2* and/or *KIR2DL2*, and/or *2DL5B* and/or centromeric *2DS3/5*). The telomeric A (*telA*) region was defined by *KIR3DL1* and *KIR2DS4* and absence of any A haplotype gene, and the telomeric B (*telB*) region was defined by presence of any centromeric B haplotype gene (*KIR3DS1* and/or *KIR2DS1* and/or *2DL5B* and/or telomeric *2DS5*) (Fig. 1). The *KIR* and *HLA-C* genotypes were ascertained according to the Allele Frequency Net Database (http://www.allele frequencies.net).

**KIR Copy number determination by multiplex quantitative PCR**

Copy numbers for all *KIR* genes (*KIR2DL1–5, 2DS1–5, 2DS4* (separate assays for the gene, wild-type variant [2DS4WT], and deletion variant [2DS4DEL]), 2DP1, 3DP1, 3DL1-3 and 3DS1) were determined using a Roche Light Cycler 480. Copy numbers were measured by relative quantification analysis of the target *KIR* gene and reference gene (signal transducer
and activator of transcription 6; STAT 6) using the comparative Cq method (26, 29). Cq value is the qPCR cycle at which fluorescence from amplification exceeds the background fluorescence (also referred to as threshold cycle, Ct). The ΔΔCq was used to calculate KIR copy number. The first ΔCq was calculated by the cycle threshold difference between the target and reference assay of the same sample. The second ΔCq was calculated by the difference of ΔCq values from a test sample and a calibrator sample with known copy number of the target. Two controls with known copy number and one non-template control were included in each run. COPYCALLER software from Applied Biosystems (Foster City, CA) was used to score KIR copy numbers. When the Cq of the reference gene was greater than 32 or a data point was more than 4 SD from the mean ΔCq of four replicates, the reaction was not analysed. KIR copy number frequencies were calculated for all the samples.

Statistical methods

We described the data across the 3 sites using frequencies and percentages for categorical variables. Frequencies of KIR genes, KIR genotypes and HLA-C allotypes were calculated by direct counting. Differences in the distribution of KIR and HLA-C genetic variants within the three populations were compared by Chi-square and Mid-P exact tests. A p-value < 0.05 was considered significant.

Results

Characteristics of study participants and populations

Among the 1,344 subjects in the 3 cohorts, 44% were under 5 years of age, and 56% between 5-10 years. A recent report defined malaria transmission, prevalence, and incidence in the 3 cohorts (Table 2) (23). The 3 sites differed markedly, with very high transmission intensity, parasite prevalence, and malaria incidence in Tororo District, lower levels of all of these parameters in Kanungu District, and the lowest levels in Jinja District (23, 30). Of note, malaria transmission was considerably greater in earlier surveys in Jinja District (31), with decreasing transmission likely due to the peri-urban nature of the study.
In Tororo District, transmission has subsequently decreased greatly, after annual indoor residual spraying of insecticides was launched in 2014 (32). Historically, malaria transmission intensity followed the rank order Tororo > Jinja > Kanungu (31). Our aim was to compare KIR and HLA-C genetic variants that may have been selected due to differential malaria selection pressures at these sites.

Comparative prevalence of KIR and HLA-C genetic variants at 3 sites in Uganda

We analysed for differential prevalence of KIR genes, KIR genotypes, HLA-C allotypes (HLA-C1C1, C1C2, and C2C2), centromeric and telomeric KIR motifs and KIR/HLA-C combinations across the 3 populations (Table 3). More than 90% of samples from all the study populations were successfully analysed for KIR and HLA-C genetic variants. The prevalence of the inhibitory KIR genes KIR2DL1 and KIR3DL1 and the activating gene KIR2DS4 was very high (>95%). The prevalence of KIR3DS1 was generally low across the 3 populations, with the lowest prevalence in Kanungu (7.6%) compared to Jinja (18.1%) and Tororo (13.2%). The prevalence of KIR2DS5 was lower in Kanungu (33.2%) compared to Jinja (43.5%) and Tororo (46.6%). The prevalence of HLA-C1C2 heterozygotes was higher (53.4%) in all 3 populations compared to homozygous HLA-C1 (20.1%) or homozygous HLA-C2 (26.5%).

The prevalence of KIR3DS1, 2DL5, 2DS5 and 2DS1 genes was significantly lower in Kanungu compared to both Tororo (7.6% vs. 13.2%: p=0.006, 57.2% vs. 66.4%: p=0.005, 33.2% vs. 46.6%: p<0.001 and 19.7% vs. 26.7%: p=0.014 respectively) and Jinja (7.6% vs.18.1%: p<0.001, 57.2% vs. 63.8%: p=0.048, 33.2% vs. 43.5%: p=0.002 and 19.7% vs. 30.4%: p<0.001 respectively). There was no significant difference in the prevalence of inhibitory KIR2DL1, 2DL2, 2DL3 and 3DL1 and activating KIR2DS2, 2DS3 and 2DS4 (Table 4). The prevalence of homozygous HLA-C2 was significantly higher in Kanungu (31.6%) compared to Jinja (21.4%), p=0.043. No significant difference was observed between the prevalence of HLA-C2 in Tororo (26.7%) and Kanungu (31.6%), p=0.296. There was no significant difference in the prevalence of KIR AA and KIR BX genotypes in Tororo and Jinja.
Combinations of KIR genotypes with HLA-C ligands did not differ in the 3 populations. There was no significant difference in the KIR centromeric or telomeric motifs across the 3 populations (Table 5).

Copy number variation in KIR genes and malaria transmission intensity

We examined whether CNV in KIR genes is influenced by malaria transmission intensity by comparing KIR CNV in children from the 3 populations. Comparisons were done for inhibitory KIR2DL1, 2DL2, 2DL3 and 2DL5, and the activating KIR2DS2 and 2DS5. All the KIR genes including framework genes were subject to CNV. The majority of study participants (over 90%) had 0-2 copies. However, there was no significant difference in KIR CNV across the 3 study populations (Table 6), suggesting that CNV in KIR genes may not be influenced by P. falciparum pathogen pressure.

Discussion

Interest in associations between genetic variation in KIR and HLA class I molecules and malaria has focused mainly on protection from severe malaria (33). In this study we considered whether KIR and HLA-C genetic variants and copy number variation in KIR genes from 3 populations of Uganda with historically varied malaria transmission intensity have been shaped by selection pressure from P. falciparum malaria. Appreciation of malaria transmission prior to recent intensive control efforts and urbanization suggests a rank order for historical transmission intensity of Tororo > Jinja > Kanungu (31). Thus, our measured prevalence of KIR and HLA genetic variation was expected to inform regarding impacts of malaria transmission intensity on evolution of KIR and HLA genes.

There was high KIR diversity in the 3 studied populations, as has been seen in previous studies in Uganda (20) and in other African populations (34). Generally, the frequency of KIR3DS1 was low across the 3 populations, similar to what has been reported in previous studies from other African populations (35). The frequency of KIR3DS1 was significantly lower in Kanungu compared to Tororo and Jinja, implying that KIR3DS1 could
have been positively selected for in Tororo and Jinja to offer some advantage against malaria. The prevalence of KIR2DS5 and KIR2DL5 genes was significantly lower in Kanungu. Interestingly, results from a previous study in Nigeria demonstrated that KIR2DS5 and KIR2DL5 genes were associated with reduced parasitemia (36). The KIR3DS1, KIR2DL5, KIR2DS5 and KIR2DS1 genes can be present together on a particular haplotype in sub-Saharan Africans (37). Differences in the prevalence of this haplotype across the three sites could potentially be explained by the selective pressure imposed by malaria. If so, the responsible gene or genes on the haplotype are not known, but KIR3DS1 has a low frequency and is present on few other haplotypes in Ugandans (38). This gene is more prevalent in other populations, including Europeans (39), suggesting that it is selected against in Uganda or it evolved outside Africa (35). The observed differences may be due, in part, to genetic differences between the ethnic groups principally inhabiting these regions. Indeed, in our previous study from these cohorts, we observed that the populations of Tororo and Kanungu were homogeneous, based on language groups, but the Jinja population had ethnic groups from all over Uganda (40). Although the specific ligands and expression details for KIR2DS3 and KIR2DS5 are yet to be defined, we speculate that under functionally relevant combinations these activating genes in conjunction with their putative ligands may increase the threshold of NK cell activation and subsequent recruitment of other immune factors that mediate protection against malaria.

Although we did not observe any significant differences in KIR/HLA-C combinations between Ugandan sites, it should be noted that, interactions between KIR and their HLA-C ligands within an individual play a key role in modulating the activity of NK cells (41). For instance, the presence of particular HLA-C allotypes and inhibitory KIR2DL1, KIR2DL2 and KIR2DL3 genes determines the strength of NK cell inhibition during malaria infection (33). The best characterized KIR-HLA ligand interactions are KIR2DL1 with the HLA-C2 subgroup and KIR2DL2/L3 with the HLA-C1 subgroup. Generally, KIR2DL1/HLA-C2 provides the strongest inhibition, followed by KIR2DL2/HLAC1, and KIR2DL3/HLA-C1 (42, 43). HLA-C1/C1 individuals are only able to receive inhibitory signals via KIR2DL2 and KIR2DL3,
whereas HLA-C2/C2 individuals receive inhibitory signals predominantly via KIR2DL1, and heterozygous individuals have the ligand for all three of these KIR genes (44). Lower KIR inhibition may allow unrestrained NK cell activation that could contribute to immune-mediated pathology. This would be consistent with the theory that mechanisms that prevent malaria infection and those that prevent severe disease are distinct and may have a balancing effect on the maintenance of different KIR and their HLA ligands in malaria endemic populations.

The association of KIR/HLA compound genotypes with malaria risk requires more attention given that malaria parasites spend most of the life cycle outside of HLA-expressing cells. Sporozoites infect hepatocytes after injection by mosquitoes. This is the only stage in the parasite replicative life cycle which is within an HLA-expressing host cell (39). Because erythrocyte membranes contain little to no HLA (42), we postulate that the influence of KIR on cell-mediated anti-parasite immunity may occur primarily during the liver stage. This implies that cellular immune responses play an important role in restricting P. falciparum infection. During the blood stage, KIR-expressing effector cells may respond more strongly to an HLA-devoid cell due to the loss of inhibitory signalling via inhibitory KIR (43). KIR inhibition may also influence the clearance of parasites through antibody-dependent cellular cytotoxicity (44, 45).

Previous studies have indicated that variation in KIR copy number, which leads to expression differences (14), may be important for susceptibility to some diseases. For example, CNV of KIR3DL1/S1 influences HIV control (45) and expression differences of KIR2DL3, interacting with HLA-C, may have a profound effect on resolution of hepatitis C virus infection (46). However, we did not observe any significant difference between KIR CNV across the three populations.

Although different KIR and HLA variants may have been selected in different populations primarily due to differential risk of malaria, the role of other infectious pathogens that are prevalent in these malaria-endemic populations should not be overlooked, as they may also have exerted selective pressure on the evolution of KIR and HLA. Therefore, the
role of other coinfections should be considered in studies involving $KIR$ and malaria, especially in populations affected by many infectious pathogens.

This study had some limitations. First, the genotyping technique for both $KIR$ and $HLA$-$C$ could not give detailed information up to the allele level. Second, we did not look at other $HLA$ class I genes, for instance $HLA$-$B$ (e.g. $HLA$ $Bw4$ and $HLA$ $Bw6$ allotypes), which may play a role in malaria risk. Nevertheless, we analysed for $HLA$-$C$ allotypes which are the major ligands for $KIR$ genes. Despite these limitations, description of the genetic diversity of $KIR$ and their $HLA$-$C$ ligands in populations with historically varied malaria transmission intensity offered an opportunity to identify $KIR$ and $HLA$-$C$ genetic variants that are under positive selection and potentially important in protection against malaria.

Conclusions
Our study has provided baseline information about associations between $KIR$ and $HLA$-$C$ and historical risks of malaria in Ugandan populations. The $KIR3DS1$, $KIR2DL5$, $KIR2DS5$ and $KIR2DS1$ genes are potentially beneficial in malaria since these genes have been positively selected for in places with historically high malaria transmission intensity. This is the largest cohort ever studied investigating $KIR$, $HLA$-$C$, and malaria risk. We also offer a new high throughput real-time PCR assay for $HLA$-$C$ genotyping which will be useful in disease association studies that involve larger cohorts.

Declarations

Ethics approval and consent to participate
The cohort study that supplied samples for analysis and this specific study were approved by the Makerere University School of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, the University of California, San Francisco Committee on Human Research, and the University of Cambridge, UK Committee on Human Research. Written informed consent was obtained from study participants.
Consent for publication. Not applicable.

Availability of data and materials
The datasets utilized for this study are available from corresponding author on reasonable request.

Competing interests
O.C. had started in a role as an employee of AstraZeneca, UK, at the time of manuscript preparation. Other authors declare that they have no competing interests.

Funding
This work was supported through the DELTAS Africa Initiative (Grant no. 107743), that funded Stephen Tukwasibwe through a PhD fellowship award, and Annettee Nakimuli through a group leader award. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Science (AAS), Alliance for Accelerating Excellence in Science in Africa (AESA), and supported by the New Partnership for Africa’s Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (Grant no. 107743) and the UK government. Francesco Colucci is funded by Wellcome Trust grant 200841/Z/16/Z. The project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No. 695551) for John Trowsdale and James Traherne. Jyothi Jayaraman is a recipient of a fellowship from the Centre for Trophoblast Research. JIN is supported by the Fogarty International Center (Emerging Global Leader Award grant number K43TW010365). EA is supported by the Fogarty International Center of the National Institutes of Health under Award Number D43TW010526. This study was partly supported by funding from the National Institutes of Health (AI075045, AI089674, TW009343, and TW007375). The views expressed in this publication are those of the authors and not necessarily those of the funding bodies.

Authors’ contributions
JIN, JR, EA, PJ, MRK, and GD directed the clinical study that provided samples for analysis. ST, SLN, AN, AM, SC, FC, JT and PJR conceived the study design. ST, JT, OC, AM, WJ and JJ designed and carried out the reported laboratory studies. ST, FM, GA and OC performed the data analysis. All authors contributed to the preparation of this manuscript and approval of its content. All authors read and approved the final manuscript.

Acknowledgements

We thank cohort study participants and their parents and guardians; the cohort study team; and the staffs of the Infectious Diseases Research Collaboration, the MUII-Plus Monitoring and Evaluation team and the Makerere/UVRI infection and immunity program for administrative and technical support.

Figure 1. KIR haplotypes

Figure 1. KIR haplotypes: KIR A haplotypes A and B are present in all populations worldwide. KIR A haplotype is composed of mainly inhibitory KIR except KIR2DS4. Allelic polymorphism is very high in the KIR A haplotype (KIR3DL1, 3DL2 and 3DL3 exhibit > 100 alleles, and 2DL1 and 2DL3 exhibit ~ 50 alleles). Haplotypes B has several activating receptors, with variable number of genes and less allelic polymorphisms. Some KIR B haplotypes are composed of combinations of haplotypes A and B (CenA-TelB, CenB-TelA). The HLA epitopes bound by some KIRs are known and are indicated as C1, C2, or Bw4.

Table 1. HLA-C primers for high throughput qPCR
| Primer name | Sequence          |
|-------------|-------------------|
| C1Fa        | GCCGCGAGTCCAAGAGG |
| C1Fb        | GCCGCGAGTCCGAGAGG |
| C2F         | CTGACCGAGTGAACCTGCGGAAA |
| C2R         | GGAGATGGGGAAGGCTCCCCAC |
| C1R         | GCGCAGGTTCCGCAGGG |

Table 2. Characteristics of study participants and populations.

| Characteristics of sites | Tororo          | Jinja           | Kanungu         |
|--------------------------|-----------------|-----------------|-----------------|
| Location                 | South-eastern   | South-central   | South-western   |
| Setting                  | Rural           | Peri-urban      | Rural           |
| Altitude                 | 695-1443 m      | 1102-1500 m     | 886-1329 m      |

Number of study subjects

- Children below 5 years: Tororo 340, Jinja 321, Kanungu 365
- Children 5-10 years: Tororo 106, Jinja 114, Kanungu 98
- Total: 446, 435, 463

Malaria indicators (children)$^{1}$

- Entomological inoculation rate per year: Tororo 310, Jinja 2.8, Kanungu 32.0
- Parasite prevalence: 28.7%, 7.4%, 9.3%
- Malaria incidence per year: 2.81, 0.43, 1.43

$^{1}$Determined August 2011-September 2013 (23).

Table 3. Distribution of KIR and HLA-C genetic variants at the 3 sites in Uganda.

| GENETIC VARIANTS | STUDY SITES |
|------------------|-------------|
|                 | Tororo, N=438 n (%) | Jinja, N=414 n (%) | Kanungu, N=446 n (%) |
| **KIR GENES**    |              |                 |                   |
| 2DS2             | 224 (51.1)   | 218 (52.7)      | 228 (51.1)        |
| 2DL2             | 254 (58.0)   | 240 (58.0)      | 254 (57.0)        |
| 2DL3             | 366 (83.6)   | 352 (85.0)      | 380 (85.2)        |
| 2DP1             | 432 (98.6)   | 409 (98.8)      | 442 (99.1)        |
| 2DL5             | 291 (66.4)   | 264 (63.8)      | 255 (57.2)        |
| 2DS5             | 204 (46.6)   | 180 (43.5)      | 148 (33.2)        |
| 2DS3             | 92 (21.0)    | 84 (20.3)       | 111 (24.9)        |
| 2DS1             | 117 (26.7)   | 126 (30.4)      | 88 (19.7)         |
| 2DS4             | 424 (96.8)   | 392 (94.7)      | 438 (98.2)        |

| KIR GENOTYPES | STUDY SITES |
|---------------|-------------|
|               | Tororo, N=385 n (%) | Jinja, N=392 n (%) | Kanungu, N=433 n (%) |
| AA            | 150 (39.0)    | 133 (33.9)       | 171 (39.5)         |
| BX            | 235 (61.0)    | 259 (66.1)       | 262 (60.5)         |
| **HLA-C ALLOTYPES** | Tororo, N=356 | Jinja, N=168 | Kanungu, N=405 |
|---------------------|---------------|--------------|---------------|
|                     | n (%)         | n (%)        | n (%)         |
| C1C1                | 66 (18.5)     | 39 (23.2)    | 75 (18.5)     |
| C1C2                | 195 (54.8)    | 93 (55.4)    | 202 (49.9)    |
| C2C2                | 95 (26.7)     | 36 (21.4)    | 128 (31.6)    |

| **CENTROMERIC KIR REGION** | Tororo, N=438 | Jinja, N=414 | Kanungu, N=446 |
|----------------------------|---------------|--------------|---------------|
|                            | n (%)         | n (%)        | n (%)         |
| C1C1                       | 191 (43.6)    | 171 (41.3)   | 190 (42.6)    |
| C1C2                       | 175 (40.0)    | 182 (44.0)   | 192 (43.1)    |
| C2C2                       | 72 (16.4)     | 61 (14.7)    | 64 (14.3)     |

| **TELOMERIC KIR REGION** | Tororo, N=438 | Jinja, N=413 | Kanungu, N=446 |
|--------------------------|---------------|--------------|---------------|
|                          | n (%)         | n (%)        | n (%)         |
| TelAA                    | 314 (71.7)    | 283 (68.5)   | 356 (79.8)    |
| TelAB                    | 115 (26.3)    | 112 (27.1)   | 83 (18.6)     |
| TelBB                    | 9 (2.0)       | 18 (4.4)     | 7 (1.6)       |

| **COMBINATIONS OF KIR HAPLOTYPES/HLA-C** | Tororo, N=313 | Jinja, N=152 | Kanungu, N=393 |
|-------------------------------------------|---------------|--------------|---------------|
|                                           | n (%)         | n (%)        | n (%)         |
| AA/C1C1                                   | 21 (6.7)      | 11 (7.3)     | 22 (5.6)      |
| AA/C1C2                                   | 62 (19.8)     | 37 (24.3)    | 93 (23.7)     |
| AA/C2C2                                   | 37 (11.8)     | 11 (7.2)     | 46 (11.7)     |
| BX/C1C1                                   | 36 (11.5)     | 23 (15.1)    | 50 (12.7)     |
| BX/C1C2                                   | 109 (34.8)    | 46 (30.3)    | 105 (26.7)    |
| BX/C2C2                                   | 48 (15.4)     | 24 (15.8)    | 77 (19.6)     |

Table 4. Comparative prevalence of KIR genes from 3 regions of Uganda with varied malaria transmission intensity.

| **STUDY SITES** | Tororo, N=356 | Jinja, N=168 | Kanungu, N=405 | Tororo, N=438 | Jinja, N=414 | Kanungu, N=446 | Mid-P p-values |
|-----------------|---------------|--------------|---------------|---------------|--------------|---------------|----------------|
| Genes           | N  | %  | N  | %  | N  | %  | T Vs J  | T vs K  | J Vs K |
| 2DS2            | 224| 51.1%| 218| 52.7%| 228| 51.1%| 0.658| 0.995| 0.652|
| 2DL2            | 254| 58.0%| 240| 58.0%| 254| 57.0%| 0.995| 0.754| 0.762|
| 2DL3            | 366| 83.6%| 352| 85.0%| 380| 85.2%| 0.558| 0.502| 0.942|
| 2DP1            | 432| 98.6%| 409| 98.8%| 442| 99.1%| 0.834| 0.506| 0.654|
| 2DL1            | 432| 98.6%| 409| 98.8%| 441| 98.9%| 0.834| 0.739| 0.906|
| 3DL1            | 421| 96.1%| 394| 95.2%| 439| 98.4%| 0.497| 0.064| 0.061|
| 3DS1            | 58 | 13.2%| 75 | 18.1%| 34 | 7.6%| 0.052| 0.006| <0.001|
| 2DL5            | 291| 66.4%| 264| 63.8%| 255| 57.2%| 0.414| 0.005| 0.048|
| 2DS5            | 204| 46.6%| 180| 43.5%| 148| 33.2%| 0.364| <0.001| 0.002|
| 2DS3            | 92 | 21.0%| 84 | 20.3%| 111| 24.9%| 0.797| 0.170| 0.108|
| 2DS1            | 117| 26.7%| 126| 30.4%| 88 | 19.7%| 0.229| 0.014| <0.001|
| 2DS4            | 424| 96.8%| 392| 94.7%| 438| 98.2%| 0.125| 0.181| 0.059|

Mid-P exact p-values for comparisons of KIR genes in Tororo vs. Jinja, Tororo vs. Kanungu and Jinja vs. Kanungu.
Table 5. Comparative prevalence of KIR and HLA-C genetic variants from 3 regions of Uganda with varied malaria transmission intensity.

| STUDY SITES | KIR genotypes | Tororo, N=385 | Jinja, N=392 | Kanungu, N=433 | p-values |
|-------------|---------------|---------------|---------------|----------------|---------|
|             | N (%)         | N (%)         | n (%)         | T Vs J         | T vs K  | J Vs K  |
| AA          | 150 39.0%     | 133 33.9%     | 171 39.5%     | 0.145          | 0.877   | 0.098   |
| BX          | 235 61.0%     | 259 66.1%     | 262 60.5%     |                |         |         |
| HLA-C       |               |               |               |                |         |         |
| Tororo, N=356 |             | Jinja, N=168  | Kanungu, N=405|                 |         |         |
| C1C1        | 66 18.5%      | 39 23.2%      | 75 18.5%      | 0.285          | 0.296   | 0.043   |
| C1C2        | 195 54.8%     | 93 55.4%      | 202 49.9%     |                |         |         |
| C2C2        | 95 26.7%      | 36 21.4%      | 128 31.6%     |                |         |         |
| KIRAA/HLA-C |               |               |               |                |         |         |
| Tororo, N=120 |             | Jinja, N=59   | Kanungu, N=161|                 |         |         |
| AA/C1C1     | 21 17.5%      | 11 18.6%      | 22 13.7%      | 0.213          | 0.537   | 0.282   |
| AA/C1C2     | 62 51.7%      | 37 62.8%      | 93 57.8%      |                |         |         |
| AA/C2C2     | 37 30.8%      | 11 18.6%      | 46 28.5%      |                |         |         |
| KIRBX/HLA-C |               |               |               |                |         |         |
| Tororo, N=193 |             | Jinja, N=93   | Kanungu, N=232|                 |         |         |
| BX/C1C1     | 36 18.6%      | 23 24.7%      | 50 21.5%      | 0.424          | 0.062   | 0.424   |
| BX/C1C2     | 109 56.5%     | 46 49.5%      | 105 45.3%     |                |         |         |
| BX/C2C2     | 48 24.9%      | 24 25.8%      | 77 33.2%      |                |         |         |
| Centromeric KIR |   |               |               |                |         |         |
| Tororo, N=438 |             | Jinja, N=414  | Kanungu, N=446|                 |         |         |
| CenAA       | 191 43.6%     | 171 41.3%     | 190 42.6%     | 0.478          | 0.552   | 0.928   |
| CenAB       | 175 40.0%     | 182 44.0%     | 192 43.0%     |                |         |         |
| CenBB       | 72 16.4%      | 61 14.7%      | 64 14.4%      |                |         |         |
| Telomeric KIR |               |               |               |                |         |         |
| Tororo, N=438 |             | Jinja, N=413  | Kanungu, N=446|                 |         |         |
| TelAA       | 314 71.7%     | 283 68.5%     | 326 73.1%     | 0.141          | 0.088   | 0.061   |
| TelAB       | 115 26.3%     | 112 27.1%     | 113 25.3%     |                |         |         |
| TelBB       | 9 2.0%        | 18 4.4%       | 7 1.6%        |                |         |         |

P-values for comparisons of the prevalence of KIR AA vs. BX genotypes, HLA (C1C1) vs. C1C2 vs. C2C2, KIR/HLA (AA/C1C1) vs. AA/C1C2 vs. AA/C2C2, KIR/HLA (BX/C1C1) vs. BX/C1C2 vs. BX/C2C2, centromeric (CenAA) vs. CenAB vs. CenBB, telomeric (TelAA) vs. TelAB vs. TelBB in Tororo (T), Jinja (J), and Kanungu (K) districts were determined using Fisher’s exact test.

Table 6. Association between CNV in KIR genes and malaria transmission intensity
CNV is copy number variation of KIR genes. The value can be 0, 1 or 2 in these populations, F is the frequency of participants with the different copies of KIR genes.

| KIR2DL1 CNV | Tororo |  | Jinja |  | Kanungu |  | P-value |
|-------------|--------|---|-------|---|---------|---|---------|
| 0           | 6      | 1.4% | 5     | 1.2% | 5       | 1.2% | 0.985   |
| 1           | 104    | 23.7%| 99    | 23.9%| 101     | 22.6%|         |
| 2           | 328    | 74.9%| 310   | 74.9%| 340     | 76.2%|         |

| KIR2DL2 CNV | Tororo |  | Jinja |  | Kanungu |  | P-value |
|-------------|--------|---|-------|---|---------|---|---------|
| 0           | 184    | 42%  | 174   | 42%  | 192     | 43.1%| 0.834   |
| 1           | 220    | 50.2%| 199   | 48.1%| 216     | 48.4%|         |
| 2           | 34     | 7.8%  | 41    | 9.9%  | 38      | 8.5% |         |

| KIR2DL3 CNV | Tororo |  | Jinja |  | Kanungu |  | P-value |
|-------------|--------|---|-------|---|---------|---|---------|
| 0           | 72     | 16.4%| 62    | 15%   | 66      | 14.8%| 0.195   |
| 1           | 154    | 35.2%| 171   | 41.3%| 191     | 42.8%|         |
| 2           | 212    | 48.4%| 181   | 43.7%| 189     | 42.4%|         |

| KIR2DS2 CNV | Tororo |  | Jinja |  | Kanungu |  | P-value |
|-------------|--------|---|-------|---|---------|---|---------|
| 0           | 214    | 48.8%| 196   | 47.3%| 218     | 48.9%|         |
| 1           | 207    | 47.3%| 189   | 45.7%| 204     | 45.7%|         |
| 2           | 17     | 3.9%  | 29    | 7%    | 24      | 5.4% |         |

| KIR2DS5 CNV | Tororo |  | Jinja |  | Kanungu |  | P-value |
|-------------|--------|---|-------|---|---------|---|---------|
| 0           | 336    | 76.7%| 324   | 78.3%| 372     | 83.4%|         |
| 1           | 98     | 22.3%| 86    | 20.7%| 71      | 16%  |         |
| 2           | 4      | 1%   | 4     | 1%   | 3       | 0.6% |         |

| KIR2DL5 CNV | Tororo |  | Jinja |  | Kanungu |  | P-value |
|-------------|--------|---|-------|---|---------|---|---------|
| 0           | 292    | 66.8%| 282   | 68.2%| 318     | 71.4%| 0.650   |
| 1           | 138    | 31.4%| 126   | 30.5%| 121     | 27%  |         |
| 2           | 8      | 1.8% | 6     | 1.3% | 7       | 1.6% |         |

CNV is copy number variation of KIR genes. The value can be 0, 1 or 2 in these populations, F is the frequency of participants with the different copies of KIR genes.

References

1. World Health Organization. World malaria report 2018. Geneva: World Health Organization; 2018.
2. Weatherall DJ. Genetic variation and susceptibility to infection: the red cell and malaria. British Journal of Haematology. 2008;141(3):276-86.
3. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. American journal of human genetics. 2005;77(2):171-92.
4. Hedrick PW. Population genetics of malaria resistance in humans. Heredity. 2011;107(4):283-304.
5. Carter R, Mendis KN. Evolutionary and Historical Aspects of the Burden of Malaria. Clinical Microbiology Reviews. 2002;15(4):564.
6. López C, Saravia C, Gómez Camacho A, Hoebeke J, Patarroyo M. Mechanisms of genetically-based resistance to malaria. Gene. 2010;467:1-12.
7. Manjurano A, Clark TG, Nadjam B, Mtove G, Wangai H, Sepulveda N, et al. Candidate Human Genetic Polymorphisms and Severe Malaria in a Tanzanian Population. PLoS One. 2012;7(10):e47463.
8. Toure O, Konate S, Sissoko S, Niangaly A, Barry A, Sall AH, et al. Candidate Polymorphisms and Severe Malaria in a Malian Population. PLoS One. 2012;7(9):e43987.
9. Band G, Le QS, Clarke GM, Kivinen K, Hubbart C, Jeffreys AE, et al. Insights into malaria susceptibility using genome-wide data on 17,000 individuals from Africa, Asia and Oceania. Nat Commun. 2019;10(1):5732.
10. Burrack KS, Hart GT, Hamilton SE. Contributions of natural killer cells to the immune response against Plasmodium. Malaria Journal. 2019;18(1):321.
11. Wolf A-S, Sherratt S, Riley EM. NK Cells: Uncertain Allies against Malaria. Frontiers in immunology. 2017;8:212-.
12. Khakoo S, Carrington M. KIR and disease: a model system or system of models? Immunological Reviews. 2006;214.
13. Hellmann I, Lim SY, Gelman RS, Letvin NL. Association of activating KIR copy number variation of NK cells with containment of SIV replication in rhesus monkeys. PLoS Pathog. 2011;7(12):e1002436.
14. Béziat V, Traherne JA, Liu LL, Jayaraman J, Enqvist M, Larsson S, et al. Influence of KIR gene copy number on natural killer cell education. Blood. 2013;121(23):4703-7.
15. Doolan DL, Dobaño C, Baird JK. Acquired Immunity to Malaria. Clinical Microbiology Reviews. 2009;22(1):13.
16. Tukwasibwe S, Nakimuli A, Traherne J, Chazara O, Jayaraman J, Trowsdale J, et al. Variations in killer-cell immunoglobulin-like receptor and human leukocyte antigen genes and immunity to malaria. Cell Mol Immunol. 2020:1-8.
17. Kulkarni S, Martin MP, Carrington M. The Yin and Yang of HLA and KIR in human disease. Semin Immunol. 2008;20(6):343-52.
18. Radwan J, Babik W, Kaufman J, Lenz TL, Winternitz J. Advances in the Evolutionary Understanding of MHC Polymorphism. Trends in Genetics. 2020;36(4):298-311.
19. Meyer D, C Aguiar VR, Bitarello BD, C Brandt DY, Nunes K. A genomic perspective on HLA evolution. Immunogenetics. 2018;70(1):5-27.
20. Nakimuli A, Chazara O, Farrell L, Hiby SE, Tukwasibwe S, Knee O, et al. Killer cell immunoglobulin-like receptor (KIR) genes and their HLA-C ligands in a Ugandan population. Immunogenetics. 2013;65(11):765-75.
21. Chazara O, Xiong S, Moffett A. Maternal KIR and fetal HLA-C: a fine balance. Journal of Leukocyte Biology. 2011;90(4):703-16.
22. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nature reviews Immunology. 2005;5(3):201-14.
23. Kamya MR, Arinaitwe E, Wanzira H, Katureebe A, Barusya C, Kigozi SP, et al. Malaria transmission, infection, and disease at three sites with varied transmission intensity in Uganda: implications for malaria control. The American journal of tropical medicine and hygiene. 2015;92(5):903-12.
24. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, et al. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. The Journal of experimental medicine. 2004;200(8):957-65.
25. Robinson JT, Thorvalzdottir H, Winckler W, Gutman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nature biotechnology. 2011;29(1):24-6.
26. Jiang W, Johnson C, Simecek N, López-Alvarez MR, Di D, Trowsdale J, et al. qKAT: a high-throughput qPCR method for KIR gene copy number and haplotype determination. Genome Medicine. 2016;8(1):99.
27. Norman PJ, Abi-Rached L, Gendzekhadze K, Hammond JA, Moesta AK, Sharma D, et al. Meiotic recombination generates rich diversity in NK cell receptor genes, alleles, and haplotypes. Genome Res. 2009;19(5):757-69.
28. Malmberg K-J, Michaëlsson J, Parham P, Ljunggren H-G. Killer Cell Immunoglobulin-like Receptor Workshop: Insights into Evolution, Genetics, Function, and Translation. Immunity. 2011;35(5):653-7.
29. Jayaraman J, Kirgizova V, Di D, Johnson C, Jiang W, Traherne JA. qKAT: Quantitative Semi-automated Typing of Killer-cell Immunoglobulin-like Receptor Genes. Journal of visualized experiments : JoVE. 2019(145).
30. Rek J, Katrak S, Obasi H, Nayebare P, Katureebe A, Kakande E, et al. Characterizing microscopic and submicroscopic malaria parasitaemia at three sites with varied transmission intensity in Uganda. Malaria Journal. 2016;15(1):470.
31. Yeka A, Gasasira A, Mpimbaza A, Achan J, Nankabirwa J, Nsobya S, et al. Malaria in Uganda: challenges to control on the long road to elimination: I. Epidemiology and current control efforts. Acta Trop. 2012;121(3):184-95.
32. Katureebe A, Zinszer K, Arinaitwe E, Rek J, Kakande E, Charland K, et al. Measures of Malaria Burden after Long-Lasting Insecticidal Net Distribution and Indoor Residual Spraying at Three Sites in Uganda: A Prospective Observational Study. PLOS Medicine. 2016;13(11):e1002167.
33. Hirayasu K, Ohashi J, Kashiwase K, Hananantachai H, Naka I, Ogawa A, et al. Significant Association of KIR2DL3-HLA-C1 Combination with Cerebral Malaria and Implications for Co-evolution of KIR and HLA. PLoS Pathog. 2012;8(3):e1002565.
34. Norman PJ, Hollenbach JA, Nemat-Gorgani N, Guethlein LA, Hilton HG, Pando MJ, et al. Co-evolution of human leukocyte antigen (HLA) class I ligands with killer-cell immunoglobulin-like receptors (KIR) in a genetically diverse population of sub-Saharan Africans. PLoS Genet. 2013;9(10):e1003938.
35. Norman PJ, Abi-Rached L, Gendzekhadze K, Korbel D, Gleimer M, Rowley D, et al. Unusual selection on the KIR3DL1/S1 natural killer cell receptor in Africans. Nat Genet. 2007;39(9):1092-9.
36. Ademola S, Amodu O, Yindom L-M, Conway D, Aka P, Bakare A, et al. Killer-cell immunoglobulin-like receptors and falciparum malaria in southwest Nigeria. Human Immunology. 2014;75.
37. Nemat-Gorgani N, Guethlein LA. Diversity of KIR, HLA Class I, and Their Interactions in Seven Populations of Sub-Saharan Africans. 2019;202(9):2636-47.
38. Nakimuli A, Chazara O, Hiby SE, Farrell L, Tukwasibwe S, Jayaraman J, et al. A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(3):845-50.
39. Körner C, Altfeld M. Role of KIR3DS1 in human diseases. Frontiers in immunology. 2012;3:326.
40. Walakira A, Tukwasibwe S, Kiggundu M, Verra F, Kakeeto P, Ruhamyankaka E, et al. Marked variation in prevalence of malaria-protective human genetic polymorphisms across Uganda. Infection, Genetics and Evolution. 2017;55:281-7.
41. Pende D, Falco M, Vitale M, Cantoni C, Vitale C, Munari E, et al. Killer Ig-Like Receptors (KIRs): Their Role in NK Cell Modulation and Developments Leading to Their Clinical Exploitation. Frontiers in immunology. 2019;10(1179).
42. Moesta AK, Norman PJ, Yawata M, Yawata N, Gleimer M, Parham P. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. Journal of immunology. 2008;180(6):3969-79.
43. Carrington M, Wang S, Martin MP, Gao X, Schifferman M, Cheng J, et al. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. The Journal of experimental medicine. 2005;201(7):1069-75.
44. Biassoni R, Falco M, Cambiaggi A, Costa P, Verdiani S, Pende D, et al. Amino acid substitutions can influence the natural killer (NK)-mediated recognition of HLA-C molecules. Role of serine-77 and lysine-80 in the target cell protection from lysis mediated by “group 2” or “group 1” NK clones. The Journal of experimental medicine. 1995;182(2):605-9.
45. Pelak K, Need AC, Fellay J, Slianna KV, Feng S, Urban TJ, et al. Copy Number Variation of KIR Genes Influences HIV-1 Control. PLOS Biology. 2011;9(11):e1001208.
46. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK Cell Inhibitory Receptor Genes in Resolving Hepatitis C Virus Infection. Science. 2004;305(5685):872.