UC Davis

UC Davis Previously Published Works

Title

Diversity of KIR, HLA Class I, and Their Interactions in Seven Populations of Sub-Saharan Africans.

Permalink

https://escholarship.org/uc/item/2d73x83h

Journal

Journal of immunology (Baltimore, Md. : 1950), 202(9)

ISSN

0022-1767

Authors

Nemat-Gorgani, Neda
Guethlein, Lisbeth A
Henn, Brenna M
et al.

Publication Date

2019-05-01

DOI

10.4049/jimmunol.1801586

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Diversity of KIR, HLA Class I, and Their Interactions in Seven Populations of Sub-Saharan Africans

Neda Nemat-Gorgani,* Lisbeth A. Guethlein,* Brenna M. Henn,† Steven J. Norberg,‡ Jacques Chiaroni,§ Martin Sikora,¶ Lluis Quintana-Murci,‖ Joanna L. Mountain,# Paul J. Norman,,†† and Peter Parham***

***HLA class I* and *KIR* sequences were determined for Dogon, Fulani, and Baka populations of western Africa, Mbuti of central Africa, and Datooga, Iraqw, and Hadza of eastern Africa. Study of 162 individuals identified 134 *HLA class I* alleles (41 *HLA-A*, 60 *HLA-B*, and 33 *HLA-C*). Common to all populations are three *HLA-C* alleles (*C1$^+$C*07:01*, *C1$^+$C*07:02*, and *C2$^+$C*06:02*) but no *HLA-A* or *-B*. Unexpectedly, no novel *HLA class I* was identified in these previously unstudied and anthropologically distinctive populations. In contrast, of 227 *KIR* detected, 22 are present in all seven populations and 28 are novel. A high diversity of *HLA A-C-B* haplotypes was observed. In six populations, most haplotypes are represented just once. But in the Hadza, a majority of haplotypes occur more than once, with 2 having high frequencies and 10 having intermediate frequencies. The centromeric (*cen*) part of the *KIR* locus exhibits an even balance between *cenA* and *cenB* in all seven populations. The telomeric (*tel*) part has an even balance of *telA* to *telB* in East Africa, but this changes across the continent to where *telB* is vestigial in West Africa. All four KIR ligands (A3/11, Bw4, C1, and C2) are present in six of the populations. *HLA* haplotypes of the Iraqw and Hadza encode two KIR ligands, whereas the other populations have an even balance between haplotypes encoding one and two KIR ligands. Individuals in these African populations have a mean of 6.8–8.4 different interactions between KIR and HLA class I, compared with 2.9–6.5 for non-Africans. *The Journal of Immunology*, 2019, 202: 2636–2647.**

The highly polymorphic MHC class I molecules of humans comprise HLA-A, -B, and -C (1). They bind peptide Ags inside cells and carry them to the cell surface. There, the complexes of peptide and MHC class I function as ligands for various receptors and coreceptors of human lymphocytes, notably NK cells and CD8 T cells (2). This pathway of Ag presentation enables killer lymphocytes to respond to cells and tissues compromised by virus infection, malignant transformation, or other forms of cellular stress. By killing infected and transformed cells, the responding lymphocytes help prevent the spread of infection or cancer, facilitating disease elimination.

Whereas most nucleated human cells express HLA-A, -B, and -C, either constitutively or cytokine induced, in the course of reproduction, HLA-C, but not HLA-A or -B, is expressed by extravillous trophoblast cells of the fetus (3). Following embryo implantation, maternal uterine NK cells cooperate with fetal extravillous trophoblast cells in remodeling maternal arteries that will provide the placenta with blood for nourishing the fetus throughout pregnancy. In these ways, HLA class I molecules contribute to two vital biological functions: reproduction and immune defense (4).

The structural variation that distinguishes the allotypes of HLA-A, -B, and -C is germline encoded and concentrated on residues of the α_1 and α_2 domains that bind the peptide or engage with NKR or TCRs. Functionally distinctive HLA class I allotypes can differ by up to 47 aa substitutions (5). As a consequence, HLA class I allotypes differ in the peptide Ags they bind and the lymphocyte receptors that engage the peptide–HLA class I complexes.

The killer cell Ig-like receptors (KIR) are a family of signaling NK cell receptors that recognize HLA class I and are principally expressed by NK cells (6, 7). Like HLA class I, KIR variation is germline encoded in a gene family that exhibits haplotypic variation in gene number and gene content, combined with allotypic variation of each *KIR* gene (8). A given *KIR* gene encodes either an inhibitory receptor or an activating receptor. The inhibitory KIR are highly polymorphic and function to educate NK cells (9), enabling them to attack infected or malignant cells with

*Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305; †Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York, NY 11794; ‡Illumina Inc., San Diego, CA 92122; §UMR 7268–Anthropologie Bio-Culturelle, Droit, Éthique et Santé, Aix-Marseille Université, l'Etablissement Français du Sang, Centre National de la Recherche Scientifique, 13344 Marseille, France; ¶Center for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, 1350 Copenhagen, Denmark; ‖Unité de Génétique Évolutive Humaine, Institut Pasteur, 75015 Paris, France; #23andMe, Inc., Mountain View, CA 94041; **Division of Biomedical Informatics and Personalized Medicine, University of Colorado, Denver, CO 80045; and ††Department of Immunology, University of Colorado, Denver, CO 80045

ORCIDs: 0000-0002-1301-8301 (L.A.G.); 0000-0003-4998-287X (B.M.H.); 0000-0002-5984-5521 (J.L.M.); 0000-0001-8370-7703 (P.J.N.).

Received for publication December 4, 2018. Accepted for publication February 13, 2019.

This work was supported by National Institutes of Health Grants AI090905 and AI17892 (to P.P.).

The sequences presented in this article have been submitted to IPD-KIR (http://www.ebi.ac.uk/ipd/kir/) and GenBank (http://www.ncbi.nlm.gov/) under accession numbers: LM656208, LM656209, LM656210, LM656211, KT023603, LM656220, LM656222, LM656221, LM656213, LM656217, LM656212, LM656215, LM656216, LM656207, LM656218, LM656219, KF941349, LM656223, KF941348, KJ535477, KJ535476, KJ535478, KF421590, KF421592, KF421591, LM656224, LM656225, and LM656226.

Address correspondence and reprint requests to Prof. Peter Parham, Stanford University School of Medicine, Sherman Fairchild Building, D157, 299 Campus Drive West, Stanford, CA 94305-5126. E-mail address: peropa@stanford.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: *cen*, centromeric; IPD, Immuno Polymorphism Database; KIR, killer cell Ig-like receptor; SSA, sub-Saharan African; *tel*, telomeric.

Copyright © 2019 by The American Association of Immunologists, Inc. 0022-1767/19/$37.50

compromised expression of HLA class I. Activating KIR exhibit considerably less polymorphism than inhibitory KIR and are now being seen to recognize complexes of HLA class I with conserved peptides of microbial pathogens (10, 11).

HLA-C is more recently evolved than HLA-A and -B. Only great apes have an HLA-C ortholog, whereas all apes and Old World monkeys have MHC-A and -B. A further distinction is that all HLA-C allotypes are recognized by inhibitory KIR, whereas that is only true for a minority of HLA-A and -B allotypes. Dimorphism at position 80 of HLA-C distinguishes the C1 epitope (N80) recognized by KIR2DL2/3 from the C2 epitope (K80) recognized by KIR2DL1. Activating KIR2DS1 and KIR2DS5 also recognize the C2 epitope. In contrast, KIR3DL1 recognizes the minority of HLA-A and -B allotypes that carry the Bw4 epitope, and KIR3DL2 recognizes only HLA-A*03 and HLA-A*11. KIR concentrate on HLA-C recognition points to the reproductive function of KIR interactions with HLA-C, and KIR have been instrumental in the evolution of HLA-C (12, 13).

Because *HLA* genes are on chromosome 6 and the *KIR* genes are on chromosome 19, these cognate ligands and receptors segregate independently in human populations. Thus, family members frequently have combinations of HLA class I ligands and KIR that educate NK cells in different ways (9). A likely benefit of this situation is that infections are less readily transmitted from one family member to another, because any pathogen that adapts to the KIR–HLA combinations in one family member will not be adapted to their relatives having different combinations of KIR and HLA. This line of argument can also apply to pathogen transmission from one family to another or from one human population to another.

To determine how KIR and HLA class I coevolve in human populations, we have studied these ligands and receptors in human populations that are anthropologically well defined. We found that the Yucpa, an indigenous population of South America, has an unusually high frequency of $C1^{+}$HLA-C. Accompanying this abundance of C1 ligands are two Yucpa-specific variants of KIR2DL3, the inhibitory C1 receptor (14). One variant is nonfunctional, whereas the other has a reduced affinity for C1. Thus, the effect of increasing C1 frequency was selection for less effective C1 receptors. In a complementary situation, the Khomani San of southern Africa have an unusually high frequency of $C2^{+}$HLA-C. Accompanying this abundance of C2 ligands are two Khomani San recombinant-specific variants of KIR2DL1, the inhibitory C2 receptor. One variant is nonfunctional and the other has a substitution in the binding site that changes the receptor's specificity from C2 to C1. In this population, the effect of increasing frequency of the C2 ligand has been to decrease the frequency of inhibitory C2 receptors and increase the frequency of inhibitory C1 receptors (15).

Archaeological analysis of the fossil record, dating back from 195,000 to 300,000 y ago, points to Africa as the continent in which modern humans first emerged (16–19). Genetic evidence supports this model (16, 17). In particular, analysis of neutral genetic markers of mitochondrial DNA and nuclear DNA has shown that Africans have greater genetic diversity than the human populations of other continents (18–21). In contrast to neutral markers, much variation in highly polymorphic HLA class I and KIR is the product of natural selection (22, 23). Previous analyses of KIR and HLA class I diversity in Africans (Ghanaians), Asians (Japanese), Oceanians (Maori), and Amerindians (Yucpa) show similar trends to the neutral markers, with African populations having the most diversity. We therefore investigated HLA class I and KIR in seven anthropologically well-defined populations representing eastern, central, and western sub-Saharan Africa for which little is known of their HLA and KIR.

Materials and Methods

Study populations

DNA samples from seven sub-Saharan African (SSA) populations were genotyped for *KIR* and *HLA class I* genes at high resolution. These samples were collected in the context of studies looking at genetic markers other than *HLA* and *KIR* genes (24–27). Genomic DNA was isolated from saliva samples collected by buccal swab (Epicentre) from 13 Iraqw and 18 Datooga individuals, as described by Knight et al. (24). These two populations reside in northern Tanzania. A third Tanzanian population we studied is the Hadza, for which DNA samples from 52 individuals were collected in the Arusha district as described (25). The Baka (Gabon) and Mbuti (Democratic Republic of Congo) are pygmy populations; DNA samples were collected from 20 and 19 individuals, respectively (26, 27). We analyzed 29 family trios (both parents and one child) from Mali in West Africa. Genomic DNA was isolated from blood samples as described (28). The results from each child were used to inform the haplotype compositions of the parents but were not included in calculating the frequencies of haplotypes and alleles. The Mali samples comprised Dogon (n = 44) and Fulani (n = 14).

We obtained *HLA class I* genotypes for 170 of the individuals typed. For four individuals, complete *HLA-A*, *-B*, and *-C* genotypes were not obtained because of technical failure.

HLA class I genotyping

The DNA samples from Iraqw, Datooga, Baka, Mbuti, and Hadza individuals were genotyped for *HLA-A*, *-B,* and *-C* at allele level, using bead-based sequence-specific oligonucleotide probes hybridization detected with a Luminex-100 instrument (Luminex, Austin, TX). The assays were performed using LABType SSO reagents (One Lambda, Canoga Park, CA).

PCR amplification and pyrosequencing

High-resolution *KIR* genotyping of DNA samples from Iraqw, Datooga, Baka, Mbuti, and Hadza individuals was performed as described (23). Specific amplicons corresponding to individual *KIR* gene exons were made and subjected to pyrosequencing using a PSQ HS 96A instrument (QIAGEN). Novel *KIR* alleles found in the African populations were validated by generating an independent PCR amplicon, cloning each allele and sequencing several clones by Sanger methodology.

Cloning and Sanger sequencing of KIR alleles

KIR-PCR amplicons were sequenced directly in each direction using BigDye Terminator Chemistry v. 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3730 Bioanalyzer (Applied Biosystems). When candidate new alleles were identified, they were cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA). Three or more clones for each candidate were sequenced, as recommended by the curators of the Immuno Polymorphism Database (IPD) (29). The sequences of validated new *KIR* alleles have been deposited in the IPD database (http://www.ebi.ac.uk/ipd/kir/). Their names and accession numbers are listed in Supplemental Fig. 1M.

Analysis of HLA and KIR genes in Mali family trios by Illumina next generation sequencing

A pool of oligonucleotide capture probes was used to target the *KIR* genomic region and *HLA* genes for high throughput sequencing (30), with modifications to the library preparation as described (31). The fragments captured were subjected to paired-end sequencing using Illumina's MiSeq instrument and V3 sequencing chemistry (Illumina, San Diego, CA).

Processing and analysis of sequence data

The PING bioinformatics pipeline (30) was used for the assessment of *KIR* gene copy number and *KIR* allele content from the next generation sequencing data. This pipeline has the capacity to provide information on new single nucleotide polymorphisms and new recombinant *KIR* alleles.

For the Baka and Mbuti samples, the data obtained from pyrosequencing were supplemented with low-coverage (4×) whole genome sequencing data. To extract *KIR*-specific sequences, we used SAMtools 0.1.18 (32) to identify read pairs that were mapped either within the *KIR* region (hg19 coordinates: 19:55, 228, 188–55, 383, 188) or to an unallocated chromosome 19 region (GenBank: GL000209.1) corresponding to an alternative *KIR* haplotype. The *KIR* alleles present were then determined using PING (30).

The IPD (IPD/KIR database 2015 release) was used as the reference set of *KIR* alleles. *HLA-A*, *-B*, and *-C* alleles were determined using the NGSengine 1.7.0 software (GenDx, Utrecht, the Netherlands) with the

IMGT 3.18.0 combined reference set. These data were analyzed directly by the software, with no prefiltering being needed for the *HLA* genes.

KIR haplotypes

Centromeric (*cen*) and telomeric (*tel*) *KIR* haplotypes were determined separately. For the Mali samples, family trios were used to determine the *KIR* haplotypes. For other populations, the *cen* and *tel* haplotypes were deduced from the patterns of linkage disequilibrium within each population. This was achieved by first determining the most common pairs of *KIR* alleles present at adjacent genes and then choosing the most likely combination for each individual and iteration to define the entire *cen* or *tel* segment. The results obtained by this iterative approach were validated for the larger numbers of Dogon and Hadza individuals studied because we could compare them to the results obtained using PHASE. A further test of their validity was their comparison with the segregation of *cen* and *tel KIR* haplotypes in the Mali population.

Calculation of a population's mean number of HLA–KIR interactions

For each individual in a cohort, the total number of different, functional interactions between their HLA and KIR was calculated as described (31). Only ligand-receptor pairs that have been defined by functional analysis were considered (33–40). These comprise Bw4$^+$HLA-A and KIR3DL1 (33), Bw4$^+$HLA-B and KIR3DL1 (33, 35), HLA-A*03 and KIR3DL2 (36), HLA-A*11 and KIR3DL2 (37), C2$^+$HLA-C and KIR2DL1 (38), C1$^+$ and C2$^+$HLA-C and KIR2DL2 (38), C1$^+$HLA-C and KIR2DL3 (38), C2$^+$HLA-C and KIR2DS1 (38), HLA-C*16 and KIR2DS2 (40), HLA-A*11 and KIR2DS4 (34), a subset of HLA-C and KIR2DS4 (34), and C2$^+$HLA-C and KIR2DS5 (only some KIR2DS5 allotypes) (39). In counting the interactions, homozygous ligands and receptors were counted as one, and scores for each ligand-receptor pair could be 0, 1, 2, or 4.

Results

High-resolution genotyping was used to determine the allelic diversity of *KIR* and *HLA class I* genes in 180 individuals representing seven populations of SSA. The study cohorts were small, varying between 13 and 52 individuals (Supplemental Fig. 1A). Four of the populations, Dogon (Mali), Fulani (Mali), Datooga (Tanzania), and Iraqw (Tanzania), are pastoralists. The other three, Baka (Gabon), Mbuti (Democratic Republic of Congo), and Hadza (Tanzania), are hunter gatherers. The location of each of these populations is shown on the map in Fig. 1. They include populations from eastern Africa, central Africa, and western Africa. Also shown on the map are three SSA populations we previously characterized: the Ga-Adangbe (Ghana) (23), the Nama (Namibia) (31), and the Khomani San (South Africa) (31).

Every HLA class I identified in the SSA corresponds to a known allele

A total of 134 *HLA class I* alleles was identified in the 170 SSA individuals (Supplemental Fig. 1B): 41 *HLA-A* alleles, 60 *HLA-B* alleles, and 33 *HLA-C* alleles. Although the cohorts varied by up to 4-fold in size, the numbers of *HLA class I* alleles detected for each population were less variable, ranging from 34 to 55 (Supplemental Fig. 1B). None of the *HLA-A* or *HLA-B* alleles are present in all seven populations, consistent with these genes having been subject to selection by fast evolving pathogens, such as RNA viruses (41). Contrasting with *HLA-A* and *-B*, three *HLA-C* alleles are common to all seven populations; these comprise *C*06:02*, *C*07:01* and *C*07:02*. HLA-C*06:02 has the C2 epitope, whereas HLA-C*07:01 and C*07:02 have the C1 epitope (Supplemental Fig. 1B). This difference between *HLA-C* and either *HLA-A* or *-B* is consistent with *HLA-C* polymorphism having evolved more slowly than *HLA-A* and *-B* polymorphism (42), a difference attributed to its distinctive role in reproduction (4). An unanticipated finding from this study is that all 134 of the *HLA-A*, *-B*, and *-C* alleles present in these seven distinctive and unstudied SSA populations correspond to known alleles, for which the sequences were already in the HLA database (43). Thus, knowledge of the breadth and depth of *HLA class I* in SSA populations is now seen to be more complete than previously appreciated. In the combined data set for the seven SSA populations (n = 170), *HLA-A*29:01*, *HLA-B*44:03*, and *HLA-C*16:01* are, respectively, the most common *HLA-A*, *-B*, and *-C* alleles. *HLA-A*29:01* and *-B*44:03* reach the highest frequency in the Hadza (21.6 and 37.5%, respectively), whereas *HLA-C*16:01* reaches the highest frequency in the Fulani (32.1%) (Supplemental Fig. 1B).

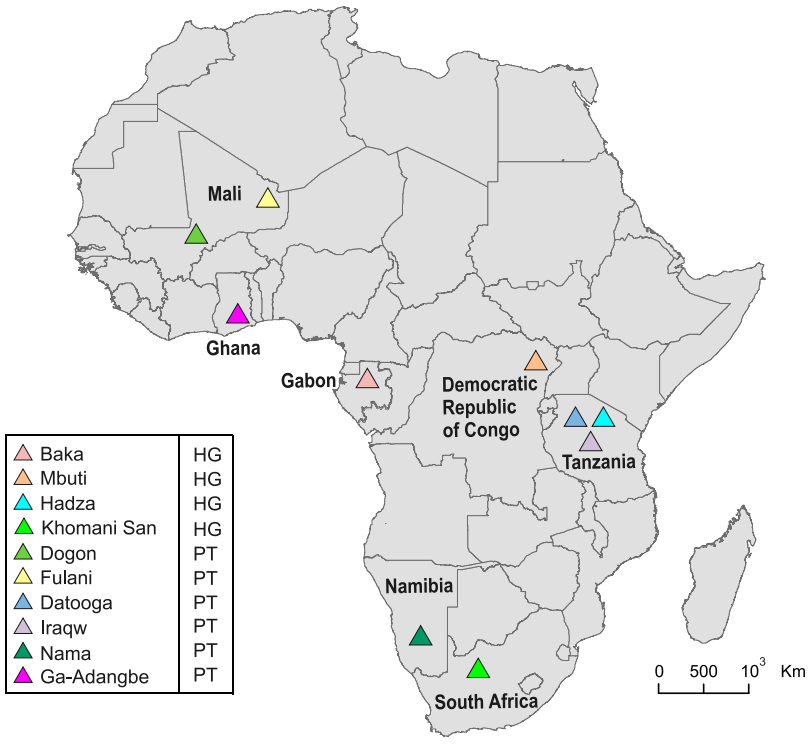


FIGURE 1. This map of Africa shows the locations of the 10 SSA populations that have been allele-level genotyped for *KIR* and *HLA class I*. Previously studied were the Ga-Adangbe from Ghana in West Africa (23) and two KhoeSan hunter-gatherer (HG) populations, the Nama and the Khomani San, from southern Africa (15, 31). Of the seven populations, on which *KIR* and *HLA class I* are, to our knowledge, first reported in this study, the Baka and Mbuti pygmies and the Hadza are hunter gatherers, and the Dogon, Fulani, Datooga, and Iraqw are pastoralists (PT).

High polymorphism and heterozygosity are characteristic features of the *HLA-A*, *-B*, and *-C* genes in all seven SSA populations (Fig. 2A). Forty-seven of the *HLA class I* alleles (35% of the total) are present in only one of the seven populations and, within this data set, are candidate "population-specific" alleles. Such alleles are present in all seven populations but to a varying degree (Supplemental Fig. 1C); a majority of them have frequencies of <0.05, with the two most common having frequencies of 0.100 (*HLA-A*66:03* in the Baka) and 0.105 (*HLA-C*08:04* in the Baka) (Supplemental Fig. 1C). Because of the small sample sizes, it is likely that some of the putative population-specific alleles are present in more than one of the populations.

Extensive diversity of HLA class I haplotypes in SSA

In the *HLA* region of the human genome, the order of the polymorphic *HLA class I* genes is *HLA-A*, *HLA-C*, and *HLA-B*. In the seven SSA populations, we defined a total of 204 different *HLA A-C-B* haplotypes. For short, these haplotypes will subsequently be called *HLA* haplotypes. Of the 204 haplotypes, 191 were observed in just one of the populations, 12 were observed in two populations, and 1 was observed in three populations (Fig. 3A). No *HLA* haplotype was seen in more than three of the seven populations (Fig. 3C, Supplemental Fig. 1D). This striking result shows how little sharing of *HLA* haplotypes there is among these anthropologically well-defined African populations. Expanding this analysis to include all African populations with defined *HLA* haplotypes (23 populations, 1134 *HLA* haplotypes) showed that 80% of the haplotypes were found only in one population. Most widespread was one haplotype present in 13 of the 23 populations (Fig. 3B). Haplotypes that are frequent in one population can be absent from the others. For example, the *HLA-A*29:02*, *-C*16:01*, *-B*78:01* haplotype has high frequency in the Fulani (21.6%) but was not detected in the other six populations (Supplemental Fig. 1D). Neither is this haplotype present in previously characterized SSA populations of western (23) and southern Africa (31). In contrast, this haplotype is present in 4% of African Americans, an admixed population with genetic input from a variety of African populations (44).

In the Mbuti, Datooga, and Iraqw cohorts, almost all *HLA* haplotypes were detected in only one individual, with three haplotypes being found in two individuals. Similarly, most Baka *HLA* haplotypes are represented once, with only six haplotypes being represented by two or three individuals (Fig. 4, Supplemental Fig. 1E). In contrast, the spectrum of Hadza *HLA* haplotype frequencies have a lower complexity. Of 88 different *HLA* haplotypes, 25 are represented only once. But two haplotypes have high frequencies, being represented 13 or 14 times. At intermediate frequencies are 10 *HLA* haplotypes, each observed in two to eight individuals (Fig. 4, Supplemental Fig. 1E). The less complex haplotype spectra observed in the Hadza supports the severe population bottleneck described by Henn et al. (25) for the population in this study. Different again is the Dogon, a population in which the majority of *HLA* haplotypes were detected in only one person. Among the minority haplotypes, one was represented 12 times, one 6 times, two 4 times, and six 2 times. Fulani *HLA* haplotypes exhibit a similar frequency spectrum to that of the Dogon, the other West African population (Fig. 4, Supplemental Fig. 1E). Thus, in six of the seven study populations, a majority (54–81%) of *HLA* haplotypes are represented once. Only in the Hadza are such singleton haplotypes a minority (28%) of the total number of *HLA* haplotypes.

To assess if this frequency spectrum of *HLA* haplotypes is a more general phenomenon, we examined cohorts representing 25 other populations, for which the cohorts studied are of similar size

A

	HLA-A		*HLA-B*		*HLA-C*		*HLA* haplotypes	
Population	*k*	H	*k*	H	*k*	H	*k*	H
Baka	18	0.92	19	0.91	14	0.88	28	0.96
Mbuti	18	0.93	16	0.90	15	0.90	29	0.96
Hadza	18	0.87	16	0.79	14	0.84	37	0.93
Dogon	19	0.89	24	0.92	12	0.86	61	0.96
Fulani	11	0.87	14	0.89	9	0.82	18	0.90
Datooga	18	0.92	22	0.95	11	0.86	29	0.96
Iraqw	16	0.91	14	0.91	10	0.86	17	0.94

B

	HLA-A		HLA-B	HLA-C	
KIR ligand	A3/11	Bw4	Bw4	C1	C2
Population	Frequency (%)				
Baka	12.5	10.0	28.9	44.7	55.3
Mbuti	5.6	19.4	50.0	44.7	55.3
Hadza	4.5	33.0	69.3	44.3	55.7
Dogon	7.0	18.0	23.0	44.0	56.0
Fulani	4.0	21.0	36.0	50.0	50.0
Datooga	13.9	8.3	50.0	43.8	56.2
Iraqw	0.0	25.0	81.8	58.3	41.7

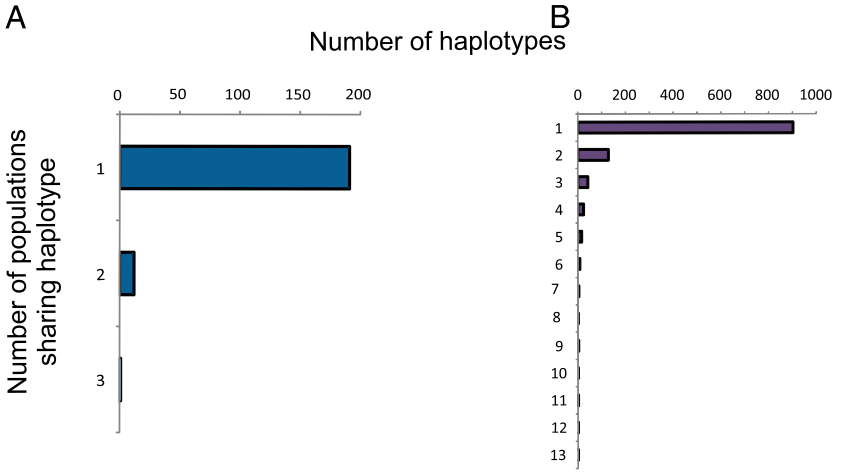
FIGURE 2. HLA class I diversity in seven SSA populations. (**A**) Shown is the variation (*k*) and heterozygosity (H) for *HLA class I* alleles and haplotypes. (**B**) The frequency of HLA-A, -B, and -C allotypes within each category of KIR ligand is shown for each population.

to the seven SSA populations. The Chihuahua Tarahumara population of Mexico has no singleton *HLA* haplotypes ($n = 44$). In contrast, 26–32% of *HLA* haplotypes are singletons in the Rukai, Yami, Puyuma, and Tsou populations of Southeast Asia. Singletons comprise 41–88% of the *HLA* haplotypes in the other 20 populations; they are particularly frequent, >80%, in populations of South African mixed ancestry, Tamil (South Asia), Mexican mixed ancestry, and Kurds (West Asia) (Fig. 4, Supplemental Fig. 1F). In conclusion, the diversity and distribution of *HLA* haplotypes we find in the seven SSA populations are also characteristic of other human populations but not all.

One mechanism that creates new *HLA* haplotypes is reciprocal recombination in the ~1300-kb region that separates *HLA-A* from *HLA-C*. In the Mbuti, for example, the five haplotypes containing *HLA-A*23:01* have different combinations of *HLA-C* and *HLA-B* alleles. Because the *HLA-C* and *HLA-B* genes are only separated by ~130 kb, the frequency of recombination between *HLA-C* and *HLA-B* is expected to be one tenth of that between *HLA-A* and *HLA-C*. Despite this constraint, we could identify 17 examples of recombination between *HLA-B* and *HLA-C* in the seven SSA populations (Supplemental Fig. 1G). In the Mbuti, two haplotypes have *HLA-A*23:01* linked to *HLA-C*02:02*. One of these haplotypes has *HLA-B*15:01*, and the other has *HLA-B*15:02*. This arrangement makes it highly likely that one of these haplotypes was derived from the other by reciprocal recombination between *HLA-B* and *HLA*-C. This difference could not have occurred through point mutation because *HLA-B*15:01* and *HLA-B*15:02* differ by 8 nt substitutions, spread throughout exons 2 and 3, that cause 5 aa substitutions in the α_1 and α_2 domains.

Hadza HLA haplotypes have a distinctive frequency spectrum

The Hadza have two high-frequency *HLA* haplotypes (*A*23:01-C*07:01-B*41:02* and *A*01:02-C*17:01-B*44:03*) that account for 31% of all Hadza *HLA* haplotypes (Supplemental Fig. 1E). That no *HLA-A*, *-B*, or *-C* allele is shared by these two haplotypes points to them having complementary functions. For example, one haplotype encodes the C1 KIR ligand (carried by HLA-C*07:01)



C

				Frequency						
	*HLA-A**	*HLA-C**	*HLA-B**	Baka	Mbuti	Hadza	Dogon	Fulani	Datooga	Iraqw
1	01:01	07:02	08:01				0.011		0.063	
2	02:02	07:02	07:02		0.063		0.011			
3	23:01	07:02	07:06				0.011	0.036		
4	23:01	02:10	15:03				0.011	0.036		
5	23:01	16:01	52:01				0.011	0.036		
6	29:01	05:01	14:01	0.028	0.031					
7	29:01	06:02	58:02	0.028	0.031					
8	30:01	17:01	08:01				0.011	0.036		
9	30:01	17:01	42:02	0.028				0.036		
10	33:01	02:02	15:01	0.028	0.031					
11	34:02	17:01	42:02		0.031			0.036		
12	36:01	04:01	53:01			0.011			0.031	0.050
13	68:02	04:01	53:01				0.011			0.100

FIGURE 3. Distribution of *HLA class I* haplotypes among seven SSA populations. (**A**) Shown are the numbers of *HLA class I* haplotypes observed in one, two, or three of the seven populations. (**B**) The numbers of haplotypes present in one, two, three, etc. up to 13 are given for all African *HLA class I* haplotypes present in the database. (**C**) Shown are *HLA class I* haplotypes found in more than one population ordered by their *HLA-A* alleles.

and the other encodes the C2 KIR ligand (carried by HLA-C*17:01). We tested Hardy–Weinberg equilibrium using three alleles; two were the high-frequency *HLA* haplotypes and the third was all other *HLA* haplotypes combined. The result of this test shows their frequencies are consistent with Hardy–Weinberg equilibrium. Consequently, the cohort of 44 Hadza includes one *C*07:01* homozygote, one *C*17:01* homozygote, and two *C*07:01/C*17:01* heterozygotes. At a moderate frequency in the Hadza are three other *HLA* haplotypes that together account for a further 20% of the *HLA* haplotypes (Supplemental Fig. 1E).

The Dogon has one common *HLA* haplotype, which is of similar frequency to the two common Hadza haplotypes. The one feature this Dogon haplotype (*A*30:01-C*17:01-B*42:01*) shares with a common Hadza haplotype is *HLA-C*17:01*, an allele characteristic of SSA populations that encodes a structurally divergent HLA-C allotype (Supplemental Fig. 1E).

The *HLA* haplotypes of the seven SSA populations were compared with those of previously studied SSA populations. These include a Ghanaian West African population (23), KhoeSan populations of southern Africa (31), and populations for which *HLA* haplotype data are deposited in the Allele Frequency Net Database (www.allelefrequencies.net/) (44, 45). Of 204 *HLA* haplotypes present in the seven SSA populations reported in this study, 28% of them are present in other SSA populations. Among these other SSA populations, the most common haplotype is *HLA-A*30:01 -C*17:01 -B*42:01* (Supplemental Fig. 1H). With a frequency of 13%, it is the most common *HLA* haplotype of the Dogon. Of the 60 different *HLA class I* haplotypes identified in the Dogon, only 26 are shared with the other SSA populations (Fig. 5A, Supplemental Fig. 1I). Most similar to the Dogon, sharing 13 haplotypes, are the Ghanaians. In contrast, the Hadza share only one haplotype (*HLA-A*36:01 -C*04:01 -B*53:01*) with the other six SSA populations reported in this study (Fig. 3C).

In summary, 73% of the *HLA class I* haplotypes in the seven SSA populations are specific to their respective population and not observed in other SSA populations. Among the seven populations, the Hadza has the most distinctive repertoire and diversity of *HLA class I* haplotypes.

Comparison of SSA HLA class I diversity to that in other populations

Comparison of the *HLA class I* diversity in populations worldwide shows the KhoeSan of southern Africa have the highest diversity (31) and Yucpa South Amerindians have the least (Fig. 5B) (46). Among the seven SSA populations, the Dogon has the highest number of different *HLA class I* alleles ($k = 55$). The Datooga has a comparable number of alleles ($k = 53$), although the cohort ($n = 16$) is much smaller than that of the Dogon ($n = 44$). Unlike most populations, in which there are more different *HLA-B* alleles than *HLA-A* alleles (14, 23, 31, 47, 48), the Iraqw, Hadza, and Mbuti have a greater number of *HLA-A* alleles than *HLA-B* alleles (Fig. 5B).

Six of seven SSA populations have the four HLA epitopes recognized by KIR

The four HLA class I epitopes recognized by KIR are the A3/11 epitope carried by HLA-A3 and HLA-A11, the Bw4 epitope carried by subsets of HLA-A and -B allotypes, and the C1 and C2 epitopes carried by subsets of HLA-C allotypes. These epitopes are also called KIR ligands. Six of the seven SSA populations have all four KIR ligands, whereas the Iraqw have Bw4, C1, and C2 but lack A3/11. That we failed to detect the A3/11 epitope in the Iraqw could be because of its absence from this population or to

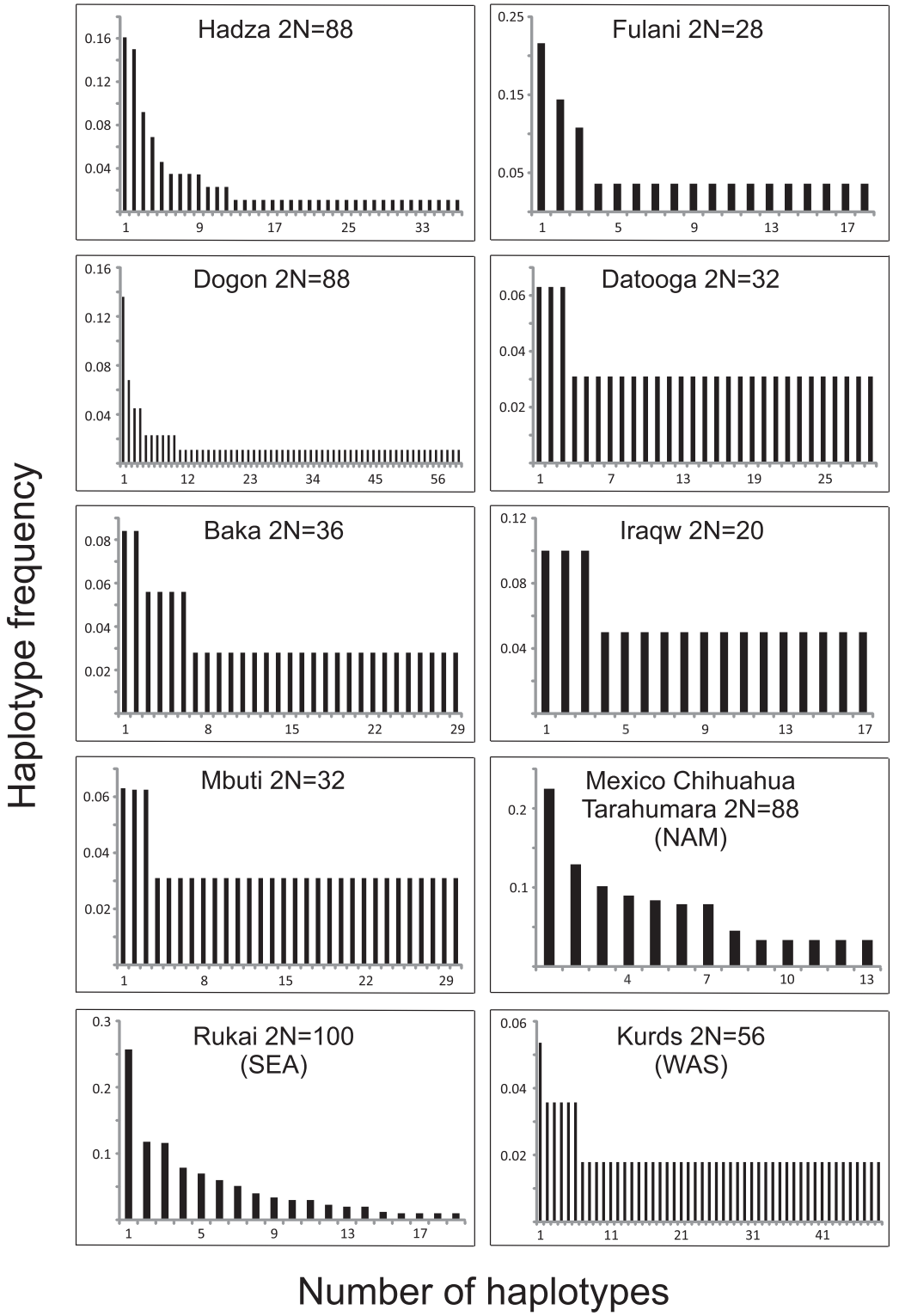
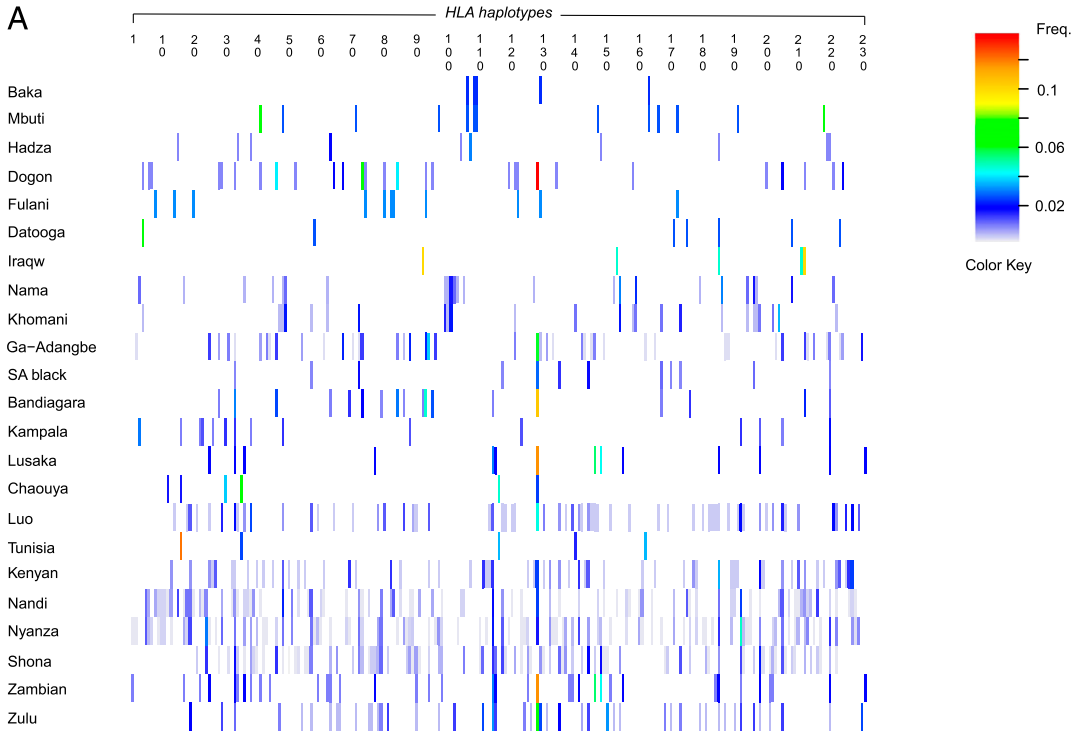


FIGURE 4. *HLA class I* haplotype diversity in seven SSA populations. Shown for each SSA population is the frequency distribution of all *HLA class I* haplotypes. Also given are the frequency distributions of *HLA class I* haplotypes in three non-African populations: North Americans (NAM), Southeast Asians (SEA), and West Asians (WAS). For each population, the total number of haplotypes (2N) is indicated.

inadequate sampling because only 10 Iraqw individuals were studied (Fig. 2B). Intriguing is the abundance in Iraqw of Bw4, which is carried by 25% of HLA-A allotypes and 82% of HLA-B allotypes. Consequently, >70% of Iraqw individuals have two or more HLA-A and -B allotypes bearing the Bw4 epitope (Supplemental Fig. 1J). In the seven SSA populations, the frequency of A3/11 ranges from 0 to 14% and that of Bw4 ranges from 8.3 to 82% (Fig. 2B). Each Hadza individual has one or more $Bw4^+$HLA-A/B allotypes and >50% of individuals have two $Bw4^+$HLA-A/B allotypes (Supplemental Fig. 1J). The largest contributor to this elevated frequency is B*44:03, possibly a consequence of selective pressure because of disease (25). Worldwide, the $Bw4^+$HLA-B allotype reaches its highest frequencies in the Hadza (69%) and the Iraqw (82%). Third in

A



B

		Number of different alleles			
Population	2N	*HLA-A*	*HLA-B*	*HLA-C*	*HLA-A,B,C*
Yucpa (SA)	122	6	7	6	19
Fulani (SSA)	28	11	14	9	34
Maori (OCE)	98	8	15	16	39
Polynesian (OCE)	68	7	17	17	41
Iraqw (SSA)	24	17	14	10	41
Hadza (SSA)	88	18	16	14	48
Mbuti (SSA)	36	18	16	15	49
Japanese (EA)	234	12	25	13	50
Baka (SSA)	40	18	19	14	51
Datooga (SSA)	36	18	24	11	53
Dogon (SSA)	88	19	24	12	55
European	182	15	25	17	57
Ghanaian (SSA)	366	26	32	23	81
Nama (SSA)	148	26	35	28	89
Khomani (SSA)	156	33	40	24	97

FIGURE 5. *HLA class I* diversity in Africans and other populations. (**A**) Heat map showing frequencies of 231 *HLA class I* haplotypes common to African populations. In the color key on the right, red denotes the highest and blue the lowest frequency haplotype. (**B**) Shown for each population are the number of *HLA-A*, *HLA-B*, and *HLA-C* alleles as well as the total number of *HLA-A*, *-B*, and *-C* alleles present in the seven SSA populations (SSA), Yucpa (South America [SA]) (14), Maori and Polynesians (Oceana [OCE]) (47), Japanese (East Asia [EA]) (48), Europeans (45), Khomani San (31), Nama (31), and Ghanaians (23). For each population, the total number of *HLA-A*, *-B*, and *-C* alleles is given.

this hierarchy is the Ga-Adangbe, another SSA population, with a Bw4$^+$HLA-B frequency of 53%. Baka combine the lowest frequency of Bw4$^+$ HLA-A/B allotypes (39%) with a relatively high frequency, 12.5%, of HLA-A allotypes having the A3/11 epitope (Fig. 2B).

Worldwide analysis identified five populations that completely lack the A3/11 epitope (Supplemental Fig. 1K). They are all indigenous Amerindian populations: the Chimila, Yucpa, and Bari of South America and the Chihuahua Tarahumara and NaDene of North America. Characterizing these populations is an abundance of the Bw4 epitope. With 58% Bw4$^+$HLA-A and 48% Bw4$^+$HLA-B, the Chimila has the highest Bw4 frequency. In contrast, the Yucpa has the lowest Bw4 frequency: 17% Bw4$^+$HLA-A and 11% Bw4$^+$HLA-B. The trend in Yucpa, for Bw4$^+$HLA-A to dominate Bw4$^+$HLA-B, increases in the Bari, who have 72% Bw4$^+$HLA-A and 6% Bw4$^+$HLA-B, and is highest in the Paiwan of Southeast

Asia who have 86% Bw4$^+$HLA-A and 26% Bw4$^+$HLA-B (Supplemental Fig. 1K).

African KIR diversity varies with geographical location

In the seven SSA populations, we detected 227 *KIR* alleles that encode 164 KIR allotypes (Fig. 6A). The individual populations have 79–113 *KIR* alleles that encode 55–77 KIR allotypes. Common to the seven populations are 22 *KIR* alleles encoding 17 KIR allotypes. These comprise 9.7% of the total *KIR*. For *KIR3DL1*, *3DL2*, and *3DL3*, the most polymorphic *KIR* genes, only seven alleles (three *3DL3*, two *3DL1*, and two *3DL2* alleles) are present in all populations, showing how each population has a different *KIR* repertoire (Supplemental Fig. 1L).

Specific to the seven SSA populations are 28 new *KIR* alleles, each specific to one of the populations (Fig. 6A). Sixteen of these *KIR* are Baka-specific, whereas the other populations have between zero and four new alleles. The Baka are a population of African pygmies, who are genetically highly diverged from the non-pygmy populations of Africans (49, 50). Thus, the abundance of new KIR alleles in the Baka is likely to be a reflection of this divergence. Defining these new alleles are 33 nt substitutions, 10 of which are silent and 23 are coding changes (Supplemental Fig. 1M). Fifteen of the new alleles were detected as single observations, and thirteen were present in two or more individuals. Most common is *KIR2DL5B*020*, which is present in 12 Hadza. Single nucleotide substitutions define 22 of the new alleles, and 6 are defined by two or more substitutions. Defining *KIR2DL5B*020* is a substitution that terminates translation at residue 283 in the cytoplasmic domain (Supplemental Fig. 1M). Although this shortens the cytoplasmic tail, the ITIM motif is retained.

Three other new *KIR* alleles have changes that are predicted to alter KIR function. Distinguishing Baka-specific *KIR2DL4*031* is a substitution that terminates translation at position 279 in an ITIM motif of the cytoplasmic tail. The second new allele, also Baka specific, is *KIR3DL1*081N*, for which a nucleotide deletion alters the reading frame in codon 85 and causes early termination. The third new allele, Hadza-specific *KIR3DL3*059*, has a substitution in codon 86, which is known to prevent cell-surface expression of KIR3DL1*004 (51). For the other 16 new *KIR* alleles that have coding changes, the substitutions are at sites that are not associated with functional change (Supplemental Fig. 1M).

For each population, the *KIR* alleles were assembled into haplotypes (see *Materials and Methods*). A total of 180 *cen* and 148 *tel KIR* haplotypes were defined (Fig. 6A, Supplemental Fig. 1N, 1O). Each of the seven populations has a relatively even balance of *cenA* and *cenB* haplotypes, in which the *cenA/B* ratio ranges from 0.9 to 2.3 (Fig. 6B). In contrast, the ratio of *telA* to *telB* haplotypes varies greatly, from 2.3 to 37, and correlates partly with geographical location (Fig. 1). In the West and central African populations, Dogon, Fulani, and Mbuti have higher *telA/B* ratios of 16.6–37; and the East African populations of Hadza, Datooga, and Iraqw have lower *telA/B* ratios of 2.3–5.0 (Fig. 6B). Exceptional is the Baka, who have a low-intermediate *telA/B* ratio (7.0). Overall, we observe a gradient from east to west across sub-Saharan Africa in which the *cenA/B* ratio remains relatively constant, whereas the *telA/B* ratio progressively increases to the point in which *telA* is dominant in West African populations and *telB* is rare. This is consistent with balancing selection operating on the *cen* region, with the *tel* region being subject to directional selection. In all seven populations, the frequencies of *cenA* and *cenB* and of *telA* and *telB* are consistent with Hardy–Weinberg equilibrium.

Common to all the sub-Saharan populations is the *cen* haplotype, *3DL3*00901-2DL3*001-2DL1*00302*. It is also the most frequent

A

	Number of KIR variants						
	Cen-A	*Cen-B*	*Tel-A*	*Tel-B*	alleles	allotypes	new alleles
Mbuti	26	12	37	1	100	70	2
Fulani	17	11	27	1	79	55	0
Dogon	54	34	83	5	113	77	1
Baka	19	21	35	5	100	76	16
Datooga	25	11	30	6	101	77	2
Hadza	61	43	75	29	105	75	4
Iraqw	15	11	18	8	96	68	3
Total	80	100	131	19	227	164	28

B

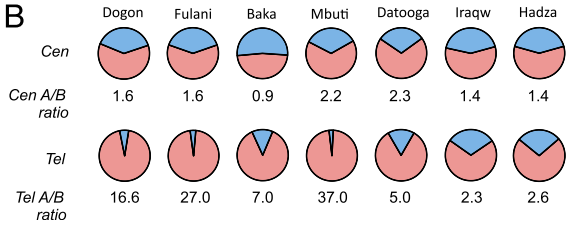


FIGURE 6. KIR diversity in seven SSA populations. (**A**) Shown are the number of *cenA*, *cenB*, *telA*, and *telB* haplotypes identified in each population. Also shown are the numbers of different *KIR* alleles, KIR allotypes, and new *KIR* alleles characterized in this study. (**B**) Shown are the frequencies of the *cenA* and *telA KIR A* haplotypes (red) and the *cenB* and *telB KIR B* haplotypes (blue) in each population, as given by the *A/B* haplotype ratio.

cen haplotype in four of the populations: Datooga, Baka, Dogon, and Fulani. This haplotype is common to populations in eastern, central, and western Africa (Fig. 7A). The dominant *cen* haplotype in the Hadza is *3DL3*019-2DL3*002-2DL1*002*. Distinguishing this haplotype is *3DL3*019*, which is absent from the other six SSA populations but present in Europeans at a frequency of 0.5%. The second most frequent *cen* haplotype of the Hadza is *3DL3*00301-2DS2*001-2DL2*00301-2DL5B*00801-2DS5*002*, which lacks the *KIR2DL1* gene. This haplotype is also present in one Iraqw individual (Fig. 7A).

No *telA* or *telB* haplotype is common to all seven SSA populations. Two common *telB* haplotypes are present in the three East African populations: Hadza (11.5% frequency), Iraqw, and Datooga (Fig. 7B). Haplotype *2DS1*002-3DL2*006* has a deletion of the *KIR2DL4-KIR2DS5* module and is common in KhoeSan (31). Haplotype *2DL4*00103-3DL1*01701-2DS4*00101-3DL2*001* has *3DL1*01701*, an African-specific allele (23).

Diversity of interaction between HLA class I and KIR in SSA

For the seven SSA populations, we determined the number of KIR ligands encoded by each HLA haplotype. For each population, we then determined the distribution of haplotypes encoding one, two, or three KIR ligands. The analysis was performed in two ways: one weighted each haplotype according to its population frequency, and the other gave each distinctive haplotype an equal weight. The two methods gave similar results (Supplemental Fig. 1P).

Although each population exhibited a different frequency distribution of *HLA* haplotypes (Fig. 8), they clearly formed two groups. For the first group (Baka, Mbuti, Dogon, Fulani, and Datooga), *HLA* haplotypes encoding one KIR ligand and *HLA* haplotypes encoding two KIR ligands have comparable frequency, whereas haplotypes encoding three KIR ligands are less frequent (Fig. 8). For the second group, comprising Iraqw and Hadza, the majority of *HLA* haplotypes encode two KIR ligands. We found no significant difference between the ligand distributions in the Iraqw and the Hadza as assessed by $\chi 2$ analysis. In the seven SSA populations, the mean number of HLA–KIR

A

	Centromeric KIR region genes						Population						
	3DL3	2DS2	2DL23	2DL5B	2DS35	2DL1	Hadz	Dato	Iraq	Baka	Mbut	Dogo	Fula
1	*00205		3*001			*00302				2	1		1
2	*00205		3*013			*002						3	1
3	*00301	*001	2*001				1	1					1
4	*00301	*001	2*001	B*00201	3*00103	*00401	1				1		
5	*00301	*001	2*001	B*00201	3*00103	*007		1	1				2
6	*00301	*001	2*003	B*00801	5*002		11		1				
7	*00501		3*001			*00302	4	2	3		5	5	3
8	*00501		3*006			*00303		1	1		2	4	
9	*00901	*001	2*001	B*00201	3*00103	*007						3	1
10	*00901		3*001			*00302	1	4	2	6	1	16	4
11	*00901		3*001			*01101	2				1		
12	*01101		3*001			*00302	8	1	1	1		6	1
13	*01101		3*006			*00302	1	1					
14	*012		3*001			*00302		1		1	1	3	
15	*01302		3*002			*002		1					1
16	*01402	*001	2*001				1	1	1				
17	*019		3*002			*002	13						
18	*035		3*00501			*006	9	1	2	1			

B

	Telomeric KIR region genes							Population						
	2DL4	3DL1S1	2DL5A	2DS5t	2DS1	2DS4	3DL2	Hadz	Dato	Iraq	Baka	Mbut	Dogo	Fula
1	*00103	*00101				*00101	*001						2	1
2	*00103	*01501				*00101	*001				1	1	1	
3	*00103	*01501				*00101	*013	6	3	2		7	5	1
4	*00103	*01502				*00101	*001		2	1	8	1	4	4
5	*00103	*01502				*00101	*029	1			1		5	2
6	*00103	*01502				*006	*001			1			1	2
7	*00103	*01502				*00101	*002	5					2	
8	*00103	*01502				*00101	*063	1	1	2				
9	*00103	*01701				*00101	*001	12	1	1				
10	*00103	*01701				*00101	*010	2	1	1			6	
11	*00103	*01701				*00101	*023	2	1	1			1	3
12	*00103	*01701				*010	*011	1		1				
13	*00103	*018				*00101	*029				2	1		
14	*00103	*020				*00101	*001	2	1					
15	*00103	*020				*00101	*009	10					2	
16	*00103	*03101				*00101	*001	2	2		1		6	1
17					*002		*006	12	1	2				
18					*002		*009			1	2			
19					*002		*019						2	1
20	*00501	S1*01301	A*00101	5*002	*002		*001	6	1					
21	*00501	S1*01301	A*00101	5*002	*002		*006	6	3					
22	*00501	S1*01301	A*00101	5*002	*002		*007	1		1				
23	*006	*007				*004	*008		2			1		
24	*00801	*00101				*003	*001		1	1		3	1	3
25	*00801	*00401				*006	*001						2	3
26	*00801	*059					*059					3	1	
27	*00802	*00401				*006	*00301		5			1		
28	*00802	*00401				*006	*00302	1				1		
29	*00802	*069				*006	*002		1	1				
30							*010	1					1	
31	*011	*00501				*010	*010	3			1	1		2
32	*01201	*022				*009	*001		1			2		

FIGURE 7. *KIR* haplotypes segregating in seven populations of SSA. Shown on the left are 18 *cen KIR* haplotypes (**A**) and 32 *tel KIR* haplotypes (**B**). Included are the most common *KIR* haplotypes present in each population and all of the *KIR* haplotypes present in two or more populations. Shown on the right are the distributions of the 50 *KIR* haplotypes among the seven SSA populations (colored boxes) and the occurrence of each haplotype in each population (the number in each box). The boxes corresponding to the most frequent *cen* and *tel KIR* haplotypes have bold outlines.

interactions per individual varied from 6.8 to 8.4, higher values than the 2.9–6.5 interactions seen in populations outside of Africa (Fig. 9A).

Discussion

Sequence analysis of *HLA-A*, *-B*, and *-C* was first applied to the common alleles of Europeans and subsequently to populations of other continents, including Africans. This led to the discovery of numerous *HLA class I* alleles that are restricted in their distribution to particular populations or groups of populations. It became a pattern that new alleles would be discovered when high-resolution molecular analysis was applied to non-white populations. For example, the indigenous populations of the Americas proved a particularly rich source of new *HLA class I* alleles (52). With this background, our expectation was that some novel *HLA class I* alleles would emerge from analysis of the anthropologically and

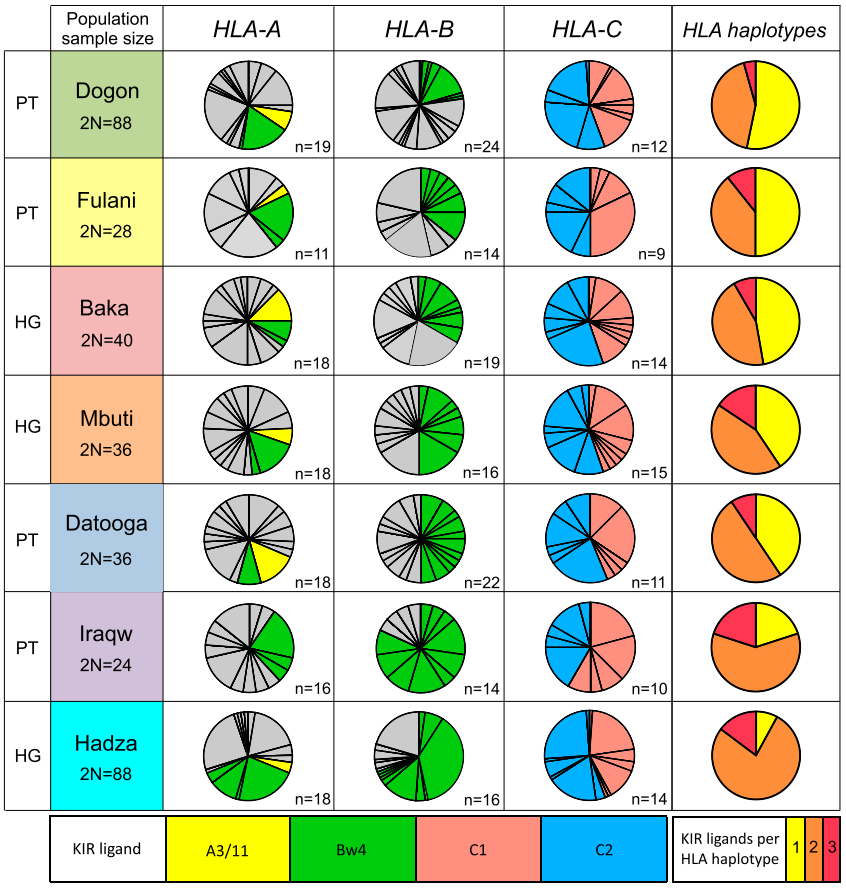


FIGURE 8. *HLA class I* allele frequency spectra for seven SSA populations. Venn diagrams (pie charts) give the frequencies of *HLA-A*, *-B*, and *-C* alleles for each population. "*n*" denotes the number of different alleles for each gene. The frequencies of allotypes having epitopes recognized by KIR are color-coded: yellow, the A3/11 epitope; green, the Bw4 epitope; red, the C1 epitope; and blue, the C2 epitope. The column on the right gives the relative frequencies of HLA class I haplotypes, encoding one (yellow), two (orange), or three (red) KIR ligands.

geographically diverse set of seven SSA populations in this study. It was therefore striking that no new HLA class I variants emerged from this study. In contrast, 28 new *KIR* were found among a total of 227 *KIR* identified in the seven populations. More than half of the new *KIR*, 16, are from the Baka population of Gabon. From this and previous studies, we can estimate a range for the numbers of *KIR* and *HLA class I* alleles that are present in anthropologically well-defined human populations. Having the least diversity is the Yucpa of South America, who have 19 *HLA class I* and 29 *KIR* alleles (14). Having the most diversity is the Nama of southern Africa, who have 97 *HLA class I* and 202 *KIR* alleles (31).

A significant finding to emerge from this investigation is the high diversity of *HLA A-C-B* haplotypes in the SSA populations. Most of the haplotypes are present in just one individual, and few haplotypes have higher frequencies. In this regard, the Hadza are exceptional because they have two haplotypes at a comparably high frequency, which account for 5% (2 of 37) of the haplotypes as well as 27% (10 of 37) of the haplotypes that have intermediate frequencies. Extending this form of analysis to other populations showed how high *HLA A-C-B* haplotype diversity is not specific to SSA populations but a general phenomenon worldwide. Among the 25 populations analyzed, 41–88% of the *HLA A-C-B* haplotypes were present in one member of the study cohort. Exceptional are four South Asian populations (Rukai, Yami, Puyuma, and Tsou) in which such singleton haplotypes are a minority and one North American population, Mexico Chihuahua Tarahumara, in which they are not found at all.

The more frequent *cen KIR* haplotypes are present in all seven SSA populations. One exception is the Hadza's most frequent *cen KIR* haplotype, which is specific to this population. In contrast, the Hadza's most frequent *tel KIR* haplotype, *2DS1*002-3DL2*006*, has a moderate frequency in the Iraqw and a highest frequency in the KhoeSan. This haplotype is specific to Africans (31).

In the SSA populations, the ratio of the *KIR A/B* haplotypes also correlates with geographical location. Whereas the ratio of *cen* haplotypes stays relatively constant across Africa, the *telA/B* ratio progressively increases from East to West, with the exception of Baka, and is lowest in the Tanzanian populations. This difference is likely the result of varying selective pressures. Disease correlation studies indicate that *telA* correlates with greater resistance to pathogens (8), whereas *telB* correlates with reproductive success (53). Possible candidates in western Africa that selected for *telA* and against *telB* include endemic pathogens, such as malaria, or any of the many rapidly evolving viruses that continually evolve to thwart the human immune response (54).

Comparing the *HLA class I* haplotypes present in the seven SSA populations with other African population haplotypes in the database revealed very little sharing among these populations, pointing again to their unique *HLA class I* diversity. Comparing this diversity with populations worldwide shows the highest diversity in the KhoeSan from southern Africa and the lowest in the Yucpa. Yucpa also lack allotypes carrying the A3/11 epitope and has the lowest frequency of $Bw4^+$ allotypes. In the SSA populations in which A3/11 is absent, there is an abundance of $Bw4^+$ epitopes. Consequently, populations with the lowest frequency of $Bw4^+$ allotypes have a relative high frequency of $A3/11^+$ allotypes. This is not always the case in other cohorts. Populations with a low frequency of $Bw4^+$ allotypes do not necessarily have a high frequency of $A3/11^+$ allotypes.

The distribution of KIR ligands is similar in most of the SSA populations (Dogon, Fulani, Baka, Mbuti, and Datooga). In these populations, a majority of *HLA* haplotypes encode one or two KIR ligands and a minority of haplotypes encode three KIR ligands.

A

Population	Mean number of HLA-KIR interactions				
	Africa	Europe	Asia	Oceania	America
Baka	8.4				
Fulani	8.1				
Khomani (31)	7.8				
Hadza	7.7				
Mbuti	7.5				
Iraqw	7.3				
Nama (31)	7.2				
Ghanaian (23)	7.0				
Datooga	6.9				
Dogon	6.8				
European		6.5			
Maori (47)				6.0	
Japanese (48)			5.2		
Polynesian (47)				4.1	
Yucpa (14)					2.9

B

Population	Genotype frequency (%)		
	AA	C2 fetus	At risk for pre-eclampsia
Baka	30.0	80.0	24.0
Mbuti	47.4	80.0	37.9
Hadza	19.2	80.4	15.5
Dogon	38.6	80.6	31.2
Fulani	50.0	75.0	37.5
Datooga	27.8	80.8	22.4
Iraqw	15.4	66.0	10.2
Nama (31)	23.2	88.9	20.6
Khomani (31)	5.2	85.1	4.4
Ga-Adangbe (23)	31.8	79.8	25.4
Kampala (55)	27.9	77.2	21.5
European (56)	29.5	57.4	16.9

FIGURE 9. Diversity in the functional potential of KIR–HLA interactions in human populations. (**A**) For SSA (23, 31) and other populations (14, 47, 48), the combined *KIR* and *HLA class I* genotype was used to define, for each individual, the total number of different pairs of interacting KIR and HLA class I ligands. Averaging the number of such interactions over the population gives the "Mean number of HLA–KIR interactions." (**B**) Pregnancies in which the mother is homozygous for C1 and KIR A, and the fetus expresses C2, are at risk for pre-eclampsia. Shown are the predicted frequencies of such pregnancies in SSA populations and Europeans (23, 31, 55, 56).

A somewhat different distribution is seen in Hadza and Iraqw, for which the majority of *HLA* haplotypes encode two KIR ligands, and the minority comprises roughly equal numbers of *HLA* haplotypes encoding one or three KIR ligands.

The Hadza has the most distinctive diversity of *HLA class I*, sharing only one haplotype with the other populations in this study. The two high-frequency haplotypes do not have any *HLA* alleles in common and likely have complementary functions. The presence of these high-frequency haplotypes in the Hadza could reflect their benefits in the immune response to infection and/or the reproductive success of individuals carrying these haplotypes.

As an assessment of the functional effect of KIR–HLA interactions, we compared the risk of pre-eclampsia among the human populations for which both *KIR* and *HLA class I* genotypes were known for each individual. Pregnancies in which the fetus expresses C2 and the mother is homozygous for C1 and *KIR cenA* are at risk for pre-eclampsia (53). This correlation suggests that inhibitory interaction between fetal C2 and maternal KIR2DL1 contributes to pre-eclampsia. This effect can be offset if the mother expresses an activating C2 receptor, which in Europeans is KIR2DS1*002 and in Africans is the subset of KIR2DS5 allotypes that recognize C2 (39, 55). In particular, 2DS5*006 is significantly associated with the protection of Ugandan women from pre-eclampsia (39). Among the seven populations studied in this study, the Mbuti and Fulani are predicted to have the highest risk of pre-eclampsia, whereas the Iraqw are predicted to have the lowest risk (Fig. 9B). To explore what might provide protection for these populations, we calculated the frequencies of their *KIR-AB* and *KIR-BB* genotypes and grouped them according to the presence of the C2 ligand as well as the activating, KIR2DS1 and KIR2DS5, and inhibitory, 2DL1, C2 receptors. The analysis suggests that protection in the Iraqw could come from KIR2DS1, which has high frequency (46.2%), whereas protection in the Baka could come from KIR2DS5, which is present in 45% of individuals having either an *AB* or *BB KIR* genotype. In the Hadza, both KIR2DS1 and KIR2DS5 could provide protection (Supplemental Fig. 1Q).

On starting this project, we expected to discover new HLA-A, -B, and -C coding sequence alleles that are specific to one or more SSA populations. Instead, we found no single allele that had not been seen before. Because Africans are genetically the most diverse of human population groups and the least well studied, our results raise the possibility that most if not all of HLA-A, -B, and -C coding sequences are now known. In the context of hematopoietic cell transplantation for genetic and malignant diseases, the next challenge is to characterize polymorphisms in noncoding regions of *HLA* genes that influence the expression and function of their protein products. The *KIR* genes also have a genetic variation and polymorphism that bears comparison with that of the *HLA* genes. In this study, 28 previously unknown KIR coding sequence alleles were defined, showing how further exploration will be needed to map the full extent of human *KIR* diversity.

Disclosures

The authors have no financial conflicts of interest.

References

1. Trowsdale, J., and J. C. Knight. 2013. Major histocompatibility complex genomics and human disease. *Annu. Rev. Genomics Hum. Genet.* 14: 301–323.
2. Cruz, F. M., J. D. Colbert, E. Merino, B. A. Kriegsman, and K. L. Rock. 2017. The biology and underlying mechanisms of cross-presentation of exogenous antigens on MHC-I molecules. *Annu. Rev. Immunol.* 35: 149–176.
3. King, A., C. Boocock, A. M. Sharkey, L. Gardner, A. Beretta, A. G. Siccardi, and Y. W. Loke. 1996. Evidence for the expression of HLAA-C class I mRNA and protein by human first trimester trophoblast. *J. Immunol.* 156: 2068–2076.
4. Parham, P., and A. Moffett. 2013. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat. Rev. Immunol.* 13: 133–144.
5. Robinson, J., L. A. Guethlein, N. Cereb, S. Y. Yang, P. J. Norman, S. G. E. Marsh, and P. Parham. 2017. Distinguishing functional polymorphism from random variation in the sequences of >10,000 HLA-A, -B and -C alleles. *PLoS Genet.* 13: e1006862.
6. Long, E. O. 2002. Tumor cell recognition by natural killer cells. *Semin. Cancer Biol.* 12: 57–61.
7. Campbell, K. S., and A. K. Purdy. 2011. Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations. *Immunology* 132: 315–325.
8. Bashirova, A. A., M. P. Martin, D. W. McVicar, and M. Carrington. 2006. The killer immunoglobulin-like receptor gene cluster: tuning the genome for defense. *Annu. Rev. Genomics Hum. Genet.* 7: 277–300.
9. Boudreau, J. E., and K. C. Hsu. 2018. Natural killer cell education and the response to infection and cancer therapy: stay tuned. *Trends Immunol.* 39: 222–239.
10. Naiyer, M. M., S. A. Cassidy, A. Magri, V. Cowton, K. Chen, S. Mansour, H. Kranidioti, B. Mbirbindi, P. Rettman, S. Harris, et al. 2017. KIR2DS2 recognizes conserved peptides derived from viral helicases in the context of HLA-C. *Sci. Immunol.* 2: eaal5296.

11. Sim, M. J., S. A. Malaker, A. Khan, J. M. Stowell, J. Shabanowitz, M. E. Peterson, S. Rajagopalan, D. F. Hunt, D. M. Altmann, E. O. Long, and R. J. Boyton. 2017. Canonical and cross-reactive binding of NK cell inhibitory receptors to HLA-C allotypes is dictated by peptides bound to HLA-C. *Front. Immunol.* 8: 193.
12. Guethlein, L. A., P. J. Norman, H. G. Hilton, and P. Parham. 2015. Co-evolution of MHC class I and variable NK cell receptors in placental mammals. *Immunol. Rev.* 267: 259–282.
13. Parham, P., and L. A. Guethlein. 2018. Genetics of natural killer cells in human health, disease, and survival. *Annu. Rev. Immunol.* 36: 519–548.
14. Gendzekhadze, K., P. J. Norman, L. Abi-Rached, T. Graef, A. K. Moesta, Z. Layrisse, and P. Parham. 2009. Co-evolution of KIR2DL3 with HLA-C in a human population retaining minimal essential diversity of KIR and HLA class I ligands. *Proc. Natl. Acad. Sci. USA* 106: 18692–18697.
15. Hilton, H. G., P. J. Norman, N. Nemat-Gorgani, A. Goyos, J. A. Hollenbach, B. M. Henn, C. R. Gignoux, L. A. Guethlein, and P. Parham. 2015. Loss and gain of natural killer cell receptor function in an African hunter-gatherer population. *PLoS Genet.* 11: e1005439.
16. Lahr, M. M., and R. A. Foley. 1998. Towards a theory of modern human origins: geography, demography, and diversity in recent human evolution. *Am. J. Phys. Anthropol.* 107(S27, Suppl. 27): 137–176.
17. Tishkoff, S. A., and S. M. Williams. 2002. Genetic analysis of African populations: human evolution and complex disease. *Nat. Rev. Genet.* 3: 611–621.
18. Chen, Y. S., A. Torroni, L. Excoffier, A. S. Santachiara-Benerecetti, and D. C. Wallace. 1995. Analysis of mtDNA variation in African populations reveals the most ancient of all human continent-specific haplogroups. *Am. J. Hum. Genet.* 57: 133–149.
19. Ingman, M., H. Kaessmann, S. Pääbo, and U. Gyllensten. 2000. Mitochondrial genome variation and the origin of modern humans. *Nature* 408: 708–713.
20. Jorde, L. B., W. S. Watkins, M. J. Bamshad, M. E. Dixon, C. E. Ricker, M. T. Seielstad, and M. A. Batzer. 2000. The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. *Am. J. Hum. Genet.* 66: 979–988.
21. Watson, E., P. Forster, M. Richards, and H. J. Bandelt. 1997. Mitochondrial footprints of human expansions in Africa. *Am. J. Hum. Genet.* 61: 691–704.
22. Norman, P. J., L. Abi-Rached, K. Gendzekhadze, D. Korbel, M. Gleimer, D. Rowley, D. Bruno, C. V. Carrington, D. Chandanayingyong, Y. H. Chang, et al. 2007. Unusual selection on the KIR3DL1/S1 natural killer cell receptor in Africans. *Nat. Genet.* 39: 1092–1099.
23. Norman, P. J., J. A. Hollenbach, N. Nemat-Gorgani, L. A. Guethlein, H. G. Hilton, M. J. Pando, K. A. Koram, E. M. Riley, L. Abi-Rached, and P. Parham. 2013. 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.* 9: e1003938.
24. Knight, A., P. A. Underhill, H. M. Mortensen, L. A. Zhivotovsky, A. A. Lin, B. M. Henn, D. Louis, M. Ruhlen, and J. L. Mountain. 2003. African Y chromosome and mtDNA divergence provides insight into the history of click languages. *Curr. Biol.* 13: 464–473.
25. Henn, B. M., C. R. Gignoux, M. Jobin, J. M. Granka, J. M. Macpherson, J. M. Kidd, L. Rodríguez-Botigué, S. Ramachandran, L. Hon, A. Brisbin, et al. 2011. Hunter-gatherer genomic diversity suggests a southern African origin for modern humans. *Proc. Natl. Acad. Sci. USA* 108: 5154–5162.
26. Cann, H. M., C. de Toma, L. Cazes, M. F. Legrand, V. Morel, L. Piouffre, J. Bodmer, W. F. Bodmer, B. Bonne-Tamir, A. Cambon-Thomsen, et al. 2002. A human genome diversity cell line panel. *Science* 296: 261–262.
27. Patin, E., K. J. Siddle, G. Laval, H. Quach, C. Harmant, N. Becker, A. Froment, B. Régnault, L. Lemée, S. Gravel, et al. 2014. The impact of agricultural emergence on the genetic history of African rainforest hunter-gatherers and agriculturalists. *Nat. Commun.* 5: 3163.
28. Ba, A., S. Beley, J. Chiaroni, P. Bailly, and M. Silvy. 2015. RH diversity in Mali: characterization of a new haplotype RHD*DIVa/RHCE*ceTI(D2). *Transfusion* 55: 1423–1431.
29. Robinson, J., J. A. Halliwell, H. McWilliam, R. Lopez, and S. G. Marsh. 2013. IPD--the immuno polymorphism database. *Nucleic Acids Res.* 41(Database issue): D1234–D1240.
30. Norman, P. J., J. A. Hollenbach, N. Nemat-Gorgani, W. M. Marin, S. J. Norberg, E. Ashouri, J. Jayaraman, E. E. Wroblewski, J. Trowsdale, R. Rajalingam, et al. 2016. Defining KIR and HLA class I genotypes at highest resolution via high-throughput sequencing. *Am. J. Hum. Genet.* 99: 375–391.
31. Nemat-Gorgani, N., H. G. Hilton, B. M. Henn, M. Lin, C. R. Gignoux, J. W. Myrick, C. J. Werely, J. M. Granka, M. Möller, E. G. Hoal, et al. 2018. Different selected mechanisms attenuated the inhibitory interaction of KIR2DL1 with C2+ HLA-C in two indigenous human populations in southern Africa. *J. Immunol.* 200: 2640–2655.
32. Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25: 2078–2079.
33. Foley, B. A., D. De Santis, E. Van Beelen, L. J. Lathbury, F. T. Christiansen, and C. S. Witt. 2008. The reactivity of Bw4+ HLA-B and HLA-A alleles with KIR3DL1: implications for patient and donor suitability for haploidentical stem cell transplantations. *Blood* 112: 435–443.
34. Graef, T., A. K. Moesta, P. J. Norman, L. Abi-Rached, L. Vago, A. M. Older Aguilar, M. Gleimer, J. A. Hammond, L. A. Guethlein, D. A. Bushnell, et al. 2009. KIR2DS4 is a product of gene conversion with KIR3DL2 that introduced specificity for HLA-A*11 while diminishing avidity for HLA-C. *J. Exp. Med.* 206: 2557–2572.
35. Gumperz, J. E., V. Litwin, J. H. Phillips, L. L. Lanier, and P. Parham. 1995. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. *J. Exp. Med.* 181: 1133–1144.
36. Döhring, C., D. Scheidegger, J. Samaridis, M. Cella, and M. Colonna. 1996. A human killer inhibitory receptor specific for HLA-A1,2. *J. Immunol.* 156: 3098–3101.
37. Hansasuta, P., T. Dong, H. Thananchai, M. Weekes, C. Willberg, H. Aldemir, S. Rowland-Jones, and V. M. Braud. 2004. Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. *Eur. J. Immunol.* 34: 1673–1679.
38. Hilton, H. G., L. A. Guethlein, A. Goyos, N. Nemat-Gorgani, D. A. Bushnell, P. J. Norman, and P. Parham. 2015. Polymorphic HLA-C receptors balance the functional characteristics of KIR haplotypes. *J. Immunol.* 195: 3160–3170.
39. Blokhuis, J. H., H. G. Hilton, L. A. Guethlein, P. J. Norman, N. Nemat-Gorgani, A. Nakimuli, O. Chazara, A. Moffett, and P. Parham. 2017. KIR2DS5 allotypes that recognize the C2 epitope of HLA-C are common among Africans and absent from Europeans. *Immun. Inflamm. Dis.* 5: 461–468.
40. Moesta, A. K., T. Graef, L. Abi-Rached, A. M. Older Aguilar, L. A. Guethlein, and P. Parham. 2010. Humans differ from other hominids in lacking an activating NK cell receptor that recognizes the C1 epitope of MHC class I. *J. Immunol.* 185: 4233–4237.
41. Martin, M. P., and M. Carrington. 2013. Immunogenetics of HIV disease. *Immunol. Rev.* 254: 245–264.
42. Buhler, S., J. M. Nunes, and A. Sanchez-Mazas. 2016. HLA class I molecular variation and peptide-binding properties suggest a model of joint divergent asymmetric selection. [Published erratum appears in 2018 *Immunogenetics* 70: 141.] *Immunogenetics* 68: 401–416.
43. Robinson, J., J. A. Halliwell, J. D. Hayhurst, P. Flicek, P. Parham, and S. G. Marsh. 2015. The IPD and IMGT/HLA database: allele variant databases. *Nucleic Acids Res.* 43(Database issue): D423–D431.
44. González-Galarza, F. F., L. Y. Takeshita, E. J. Santos, F. Kempson, M. H. Maia, A. L. da Silva, A. L. Teles e Silva, G. S. Ghattaoraya, A. Alfirevic, A. R. Jones, and D. Middleton. 2015. Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res.* 43(Database issue): D784–D788.
45. Gonzalez-Galarza, F. F., A. McCabe, E. J. Melo Dos Santos, L. Takeshita, G. Ghattaoraya, A. R. Jones, and D. Middleton. 2018. Allele frequency net database. *Methods Mol. Biol.* 1802: 49–62.
46. Gendzekhadze, K., P. J. Norman, L. Abi-Rached, Z. Layrisse, and P. Parham. 2006. High KIR diversity in Amerindians is maintained using few gene-content haplotypes. *Immunogenetics* 58: 474–480.
47. Nemat-Gorgani, N., H. A. Edinur, J. A. Hollenbach, J. A. Traherne, P. P. Dunn, G. K. Chambers, P. Parham, and P. J. Norman. 2014. KIR diversity in Māori and Polynesians: populations in which HLA-B is not a significant KIR ligand. *Immunogenetics* 66: 597–611.
48. Yawata, M., N. Yawata, M. Draghi, A. M. Little, F. Partheniou, and P. Parham. 2006. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. [Published erratum appears in 2006 *J. Exp. Med.* 203: 1131.] *J. Exp. Med.* 203: 633–645.
49. Verdu, P. 2016. African pygmies. [Published erratum appears in 2016 *Curr. Biol.* 26: 418.] *Curr. Biol.* 26: R12–R14.
50. Shriner, D., F. Tekola-Ayele, A. Adeyemo, and C. N. Rotimi. 2018. Genetic ancestry of Hadza and Sandawe peoples reveals ancient population structure in Africa. *Genome Biol. Evol.* 10: 875–882.
51. Pando, M. J., C. M. Gardiner, M. Gleimer, K. L. McQueen, and P. Parham. 2003. The protein made from a common allele of KIR3DL1 (3DL1*004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig domain 1. *J. Immunol.* 171: 6640–6649.
52. Parham, P., K. L. Arnett, E. J. Adams, A. M. Little, K. Tees, L. D. Barber, S. G. Marsh, T. Ohta, T. Markow, and M. L. Petzl-Erler. 1997. Episodic evolution and turnover of HLA-B in the indigenous human populations of the Americas. *Tissue Antigens* 50: 219–232.
53. Hiby, S. E., J. J. Walker, K. M. O'shaughnessy, C. W. Redman, M. Carrington, J. Trowsdale, and A. Moffett. 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J. Exp. Med.* 200: 957–965.
54. Rotimi, C. N., A. R. Bentley, A. P. Doumatey, G. Chen, D. Shriner, and A. Adeyemo. 2017. The genomic landscape of African populations in health and disease. *Hum. Mol. Genet.* 26(R2): R225–R236.
55. Nakimuli, A., O. Chazara, J. Byamugisha, A. M. Elliott, P. Kaleebu, F. Mirembe, and A. Moffett. 2014. Pregnancy, parturition and preeclampsia in women of African ancestry. *Am. J. Obstet. Gynecol.* 210: 510–520.e1.
56. Vierra-Green, C., D. Roe, L. Hou, C. K. Hurley, R. Rajalingam, E. Reed, T. Lebedeva, N. Yu, M. Stewart, H. Noreen, et al. 2012. Allele-level haplotype frequencies and pairwise linkage disequilibrium for 14 KIR loci in 506 European-American individuals. *PLoS One* 7: e47491.