Killer cell immunoglobulin-like receptor (*KIR*) genes and their HLA-C ligands in a Ugandan population

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Abstract Killer cell immunoglobulin-like receptor (*KIR*) genes are expressed by natural killer cells and encoded by a family of genes exhibiting considerable haplotypic and allelic variation. HLA-C molecules, the dominant ligands for KIR, are present in all individuals and are discriminated by two KIR epitopes, C1 and C2. We studied the frequencies of *KIR* genes and *HLA-C1* and *C2* groups in a large cohort (*n*=492) from Kampala, Uganda, East Africa and compared our findings with published data from other populations in sub-Saharan Africa (SSA) and several European populations. We find considerably more *KIR* diversity and weaker linkage disequilibrium in SSA compared to the European populations and describe several novel *KIR* genotypes. *C1* and *C2* frequencies were similar to other SSA populations with a higher frequency of the *C2* epitope (54.9 %) compared to Europe (average 39.7 %). Analysis of this large cohort from Uganda in the context of other African populations reveals variations in *KIR* and *HLA-C1* and *C2* that are consistent with migrations within Africa and potential selection pressures on these genes. Our results will help understand how *KIR/HLA-C* interactions contribute to resistance to pathogens and reproductive success.

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Electronic supplementary material The online version of this article (doi:10.1007/s00251-013-0724-7) contains supplementary material, which is available to authorized users.

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Introduction

Killer cell immunoglobulin-like receptor genes (*KIR*) are expressed by natural killer (NK) cells and function in infection, cancer, and reproduction. KIR bind some human leukocyte antigen (HLA) class I molecules and other unknown ligands, and a balance between activating and inhibitory signals mediated by KIR regulates NK cell functions. Both *KIR* and *HLA* genetic regions are highly variable and can be used as tools in anthropological studies (Parham and Moffett 2013) revealing the history of populations, migration, admixture, or pathogen pressure (Hedrick and Thomson 1983; Piazza et al. 1980). African populations have higher levels of genetic diversity (Tishkoff and Verrelli 2003), which has been documented by many studies using mitochondrial DNA and/or Y chromosome markers and neutral markers as well as *HLA* polymorphisms (Pilkington et al. 2008; Prugnolle et al. 2005; Tishkoff et al. 2009). *KIR* variability across the African continent is still not well documented despite the burden of infectious disease including HIV/AIDS, malaria, and tuberculosis. In addition, maternal and reproductive

health disorders are a major problem in SSA (WHO databases, www.who.int), and *KIR* and *HLA* have been associated with these diseases (Chazara et al. 2011; Hiby et al. 2010; Kulkarni et al. 2008; Parham 2005).

The *KIR* gene family is located on chromosome 19 and contains 12 genes and 2 pseudogenes (Trowsdale et al. 2001; Wende et al. 1999) The *KIR* region exhibits great diversity through both the presence and absence of genes as well as allelic polymorphism of individual genes. *KIR* nomenclature is based on the number of extracellular immunoglobulin-like domains, two domains (2D) or three domains (3D), followed by the length of their cytoplasmic tail, short or long. HLA-C is the major ligand for KIR and a dimorphism at position 80 of the α1 domain divides all HLA-C allotypes into two groups, C1 and C2 (Colonna et al. 1993; Mandelboim et al. 1996). In general, KIR2DL2 and KIR2DL3 interact with C1 allotypes, while KIR2DL1 and KIR2DS1 bind specifically to C2 allotypes.

Soon after *KIR* genes were discovered, it was appreciated that they could be subdivided into two main haplotypes, "A" and "B," distinguished by gene content (Uhrberg et al. 1997). *KIR A* haplotypes have a fixed gene content of seven genes encoding mostly inhibitory receptors, whereas *KIR B* haplotypes have a variable gene content that includes more genes encoding activating receptors. *KIR2DL4*, *3DL2*, and *3DL3* are framework genes common to both haplotypes (Wilson et al. 2000). A recombination breakpoint between *KIR2DL1* and *KIR2DL4* allows a classification of *KIR* haplotypes in Eurasians into different combinations of centromeric *A* or *B* (*cA*, *cB*) and telomeric *A* or *B* (*tA*, *tB*) regions. These regions are characterized by the presence of certain *KIR* genes: *cA* (*KIR2DL1*, *2DL3*), *cB* (*KIR2DL2*, *2DS2*), *tA* (*KIR3DL1*, *2DS4*), and *tB* (*KIR3DS1*, *2DS1*). *HLA* and *KIR* genes are the most polymorphic and variable gene systems in humans with no "wild-type" genes (Parham et al. 2011). Though *KIR* and *HLA* class I genes are located on different chromosomes (and therefore segregate independently), they have been found to be coevolving, accordingly to the receptor–ligand interactions between them (Guinan et al. 2010; Single et al. 2007).

Our aim was to investigate the variability of the *KIR* region in Africa, as the findings will have relevance to population evolutionary history, disease, and vaccine studies for populations in Africa and those with African ancestry elsewhere in the world.

Materials and methods

Study subjects

Blood samples were obtained from 492 unrelated healthy female donors at Mulago Hospital, Kampala between 2009 and 2011. The majority of individuals (61.5 %) reported one or both parent speaking the Ganda language, classified with the Bantu languages (Table S 1). The subjects were healthy with no known chronic disease, apart from a HIV prevalence of approximately 5 %. Healthy controls from the UK were used for comparison (Hiby et al. 2010). *KIR* and *HLA-C* gene frequencies and genotypes frequencies for other African populations were obtained from the Allele Frequency Net database and previous reports (Gonzalez-Galarza et al. 2011; Hollenbach et al. 2012; Omosun et al. 2012; Single et al. 2007; Yindom et al. 2010).

Ethics statement

Informed written consent was obtained, and the study was conducted under ethical approval from the Higher Degrees Research and Ethics Committee of Makerere University College of Health Sciences and the Uganda National Council for Science and Technology.

DNA isolation and genotyping

Genomic DNA was isolated from 5 mL of blood using the QIAmp DNA Maxi Blood Kit (Qiagen). Polymerase chain reaction-sequence-specific primer (PCR-SSP) was performed to amplify the genomic DNA for presence or absence of 12 *KIR* genes (*2DL1*, *2DL2*, *2DL3*, *2DL5*, *3DL1*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, and *3DS1*) and the pseudogene *2DP1*. *KIR2DS4* alleles were also typed as being either full length or having the 22-bp deletion that prevents cell surface expression. Two pairs of primers were used for each gene, selected to give relatively short amplicons of 100–800 bp, as previously

Table 1 *KIR* carrier and estimated gene frequencies in the Ugandan and UK populations

		2DS2	2DL2	2DL3	2DP1	2DL1	3DL1	3DS1	2DL5	2DS5	2DS3	2DS1	2DS4
Uganda	Carrier frequency	0.54	0.61	0.85	0.98	0.99	0.98	0.12	0.65	0.50	0.24	0.24	0.96
	Estimated gene frequency	0.32	0.37	0.62	0.87	0.88	0.86	0.06	0.41	0.29	0.13	0.13	0.79
UK	Carrier frequency	0.52	0.51	0.91	0.96	0.96	0.96	0.44	0.54	0.36	0.28	0.42	0.96
	Estimated gene frequency	0.30	0.30	0.70	0.79	0.80	0.79	0.25	0.32	0.20	0.15	0.24	0.79
Chi-square *p* value			0.00437	0.01177				<1E-07	0.00055	1.2E-05		<1E-07	

Table 2 *KIR* carrier frequencies in the Ugandan and 22 African populations

	Population	2DL1	2DL2	2DL3	2DL4	2DL5	2DP1	2DS1	2DS2	2DS3	2DS4	2DS5	3DL1	3DL2	3DL3	3DP1	3DS1	Location
Allele Frequency Net	Uganda Kampala (n=492)	0.99	0.61	0.85		0.65	0.98	0.24	0.54	0.24	0.96	0.50	0.98				0.12	0°18'N 32°34'E
	Equatorial Guinea Bioko Island Bubi (n=95)	0.96	0.57	0.80	0.99	0.52	0.97	0.20	0.48	0.26	0.97	0.34	0.99	1.00	1.00	0.99	0.05	1°55'N 10°6'E
	Gabon (n=54)	1.00	0.65	0.80	1.00	0.70	1.00	0.19	0.57	0.35	1.00	0.33	1.00	1.00	1.00	1.00	0.07	1°38'S 13°35'E
	Ghana (n=41)	0.93	0.54	0.85	1.00	0.56	1.00	0.05	0.56	0.34	0.98	0.22	1.00	1.00	1.00	1.00	0.05	5°33'N 0°15'W
	Ivory Coast Abidjan (n=25)	0.80	0.64	0.80		0.56		0.16	0.60	0.40	1.00	0.28	1.00	1.00			0.16	5°20'N 4°1'W
	Senegal (n=118)	1.00	0.55	0.90	1.00	0.52	1.00	0.13	0.42	0.24	1.00	0.30	0.99	1.00	1.00	1.00	0.04	14°0'N 16°0'W
	South Africa San (n=91)	0.96	0.74	0.60	1.00	0.68	0.92	0.14	0.73	0.25	1.00	0.64	0.99	1.00	1.00	1.00	0.02	34°21'S 18°28'E
	South Africa Xhosa (n=50)	0.96	0.72	0.64	1.00	0.82	0.98	0.10	0.64	0.38	1.00	0.62	1.00	1.00	1.00	1.00	0.04	34°0'S 19°0'E
Single et al. (2007)	Central African Republic Bagandu Biaka (n=69)	0.97	0.58	0.80				0.01	0.57				1.00				0.03	5°0'N 15°0'E
	Congo Kinshasa Ituri Forest Mbuti (n=38)	0.94	0.66	0.62				0.28	0.66				0.97				0.11	3°0'N 26°0'E
	Ethiopian (n=31)	0.90	0.81	0.77				0.26	0.77				0.97				0.23	9°1'N 38°44'E
	Nigeria Benin Yoruba (n=75)	1.00	0.38	0.92				0.15	0.35				0.99				0.12	10°0'N 2°0'E
	Nigeria Enugu Ibo (n=48)	0.96	0.61	0.76				0.08	0.58				1.00				0.06	7°0'N 5°0'E
	Nigeria Zaria Hausa (n=37)	1.00	0.37	0.97				0.14	0.32				1.00				0.03	11°4'N 7°42'E
Hollenbach et al. (2012)	Biaka Pygmies (n=29)	1.00	0.62	0.90	1.00	0.76	1.00	0.03	0.59	0.17	1.00	0.66	1.00	1.00	1.00	1.00	0.07	30–31°66–67'E
	Mandenka (n=23)	1.00	0.70	0.91	1.00	0.57	1.00	0.22	0.52	0.39	0.96	0.30	1.00	1.00	1.00	1.00	0.17	12°N 12°W
	Yoruba (n=23)	1.00	0.35	0.87	1.00	0.30	1.00	0.17	0.30	0.17	0.96	0.17	0.96	1.00	1.00	1.00	0.13	6–10°N 2–8°E
Yindom et al. (2012)	Gambia Mandingo (n=199)	1.00	0.77	0.90	1.00	0.60	1.00	0.21	0.60	0.43	1.00	0.28	1.00	1.00	1.00		0.11	13°28'N 16°36'W
	Gambia Wollof (n=88)	1.00	0.69	0.91	1.00	0.53	0.99	0.24	0.57	0.42	1.00	0.25	1.00	1.00	1.00		0.06	13°28'N 16°36'W
	Gambia Fula (n=137)	1.00	0.74	0.88	1.00	0.59	1.00	0.21	0.58	0.45	1.00	0.33	1.00	1.00	1.00		0.07	13°28'N 16°36'W
	Gambia Jola (n=89)	1.00	0.79	0.83	1.00	0.56	0.99	0.25	0.64	0.49	1.00	0.26	1.00	1.00	1.00		0.08	13°28'N 16°36'W
	Gambia Serere (n=29)	1.00	0.59	0.90	1.00	0.55	0.97	0.24	0.52	0.31	1.00	0.35	1.00	1.00	1.00		0.14	13°28'N 16°36'W
Omosun et al. (2012)	Western Kenya (predominantly Luo) (n=93)	0.94	0.57	0.84	0.99	0.61		0.20	0.50	0.25	0.91	0.40	0.96				0.11	1°16'S 36°48'E

described in full detail (Hiby et al. 2004). *HLA-C* alleles were genotyped as either group C1 or C2 allotypes. Genotyping was performed by PCR-SSP using two pairs of primers for both HLA-C allotypes, as previously detailed (Hiby et al. 2008). Extra *KIR* primers were designed using sequence information from the 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) (Robinson et al. 2011). Selected samples (based on their unusual *KIR* gene content) were typed for copy number by multiplexed quantitative PCR for all 16 *KIR* genes (*KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DP1, 3DP1, 3DL1, 3DL2, 3DL3*, and *3DS1*) (Jiang et al. 2012). *KIR* haplotypes were defined by matrix subtraction of gene copy numbers using previously characterized common and contracted *KIR*

haplotypes using the *KIR* Haplotype Identifier software (www.bioinformatics.cimr.cam.ac.uk/haplotypes).

Data analysis and statistical methods

KIR gene carrier frequencies (*F*) and *HLA-C1* and *C2* group frequencies were calculated by direct counting. *KIR* gene frequencies were estimated by the formula $f = 1 - \sqrt{(1-F)}$, where *F* is the *KIR* gene carrier frequency in the population. Differences between the Ugandan and the UK populations were compared by χ^2. A centered unscaled principal components analysis was conducted as implemented in the adegenet R package (Jombart and Ahmed 2011). A map was generated using GMT using a modified script available online (www.pypop.org/popdata, Owen Solberg) (Wessel and Smith 1991). Pairwise linkage disequilibrium (Wn*) was calculated for the
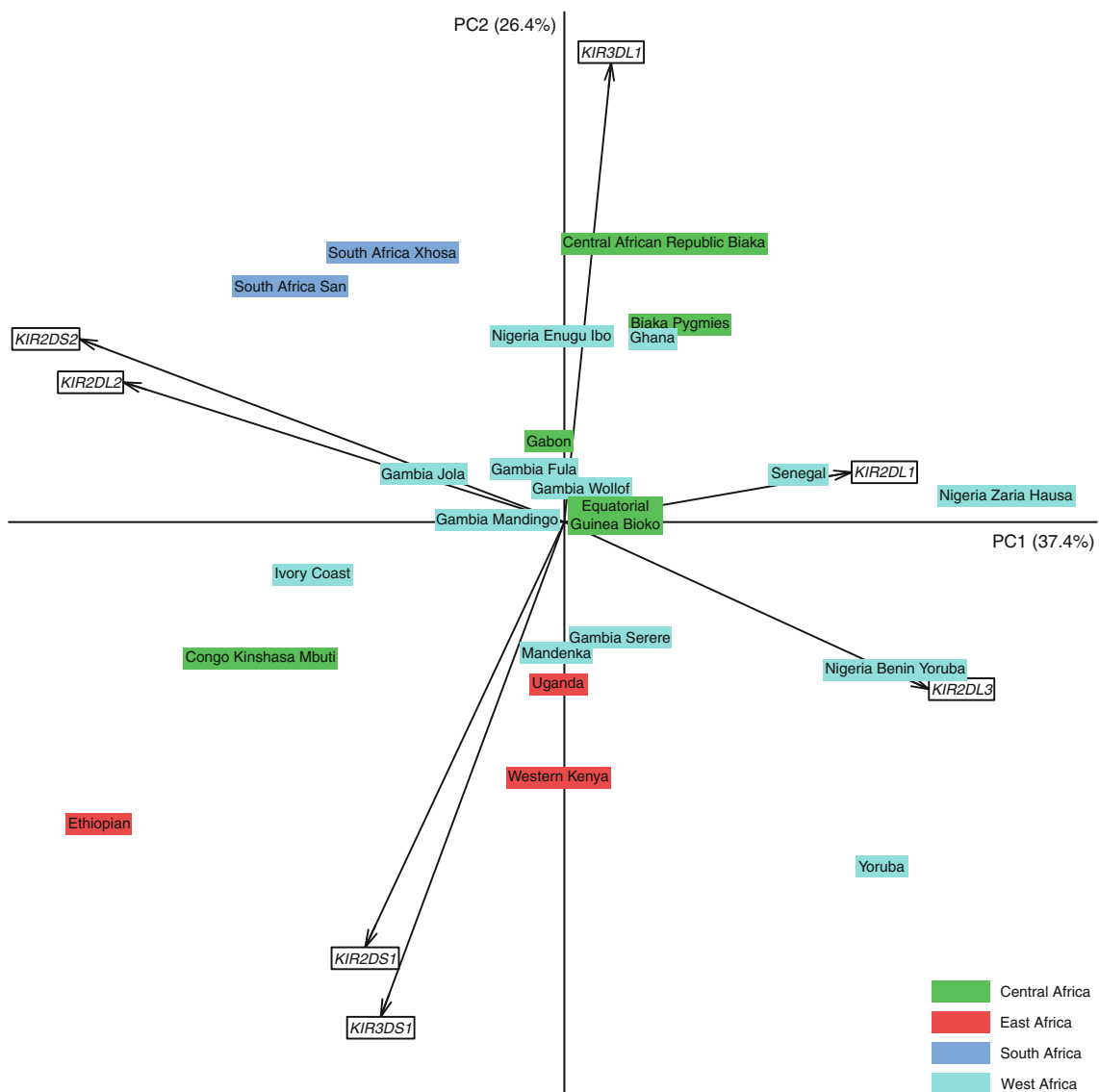
Fig. 1 Principal component analysis based on seven *KIR* gene carrier frequencies for the 23 African populations described in Table 2. *Arrows* represent the relative contribution of each *KIR* gene frequency to the variability along the first two axes (PC1 and PC2)
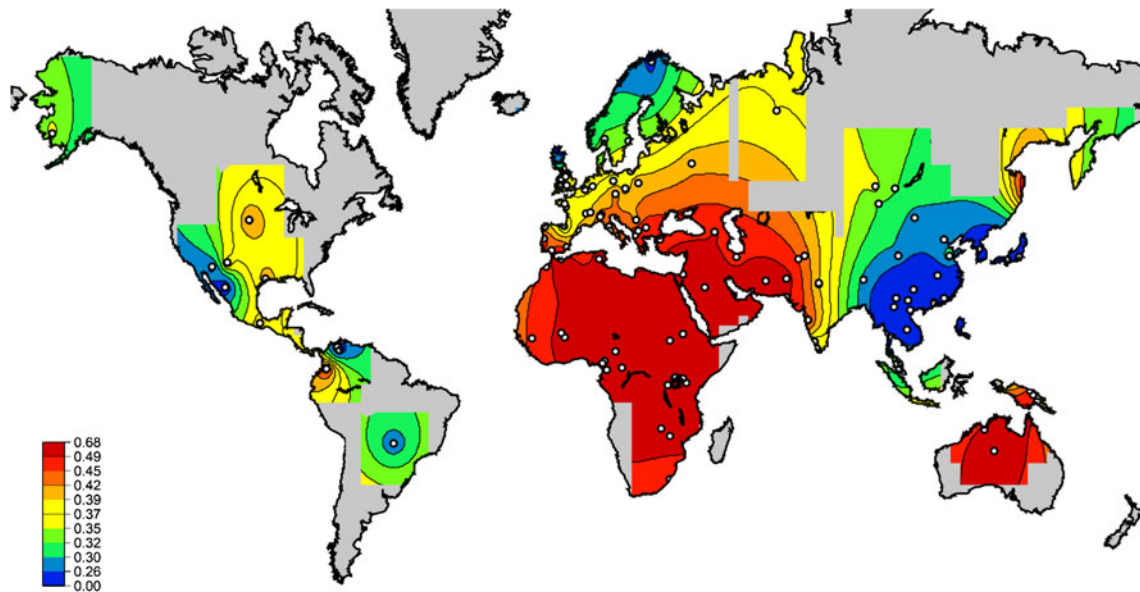
Fig. 2 Worldwide *HLA-C2* allele frequencies for 208 populations from the Allele Frequency Net database and the Ugandan population. Only non-migrant populations are included

12 *KIR* loci (Single et al. 2008). Statistical significance was obtained using Fisher's exact test, as implemented in PyPop (Lancaster et al. 2007). A graphical view of the linkage disequilibrium (LD) from our study data and that of Single et al. (2008) was achieved using Gold (Abecasis and Cookson 2000).

Results and discussion

KIR and *HLA-C* group carrier frequencies

We first compared the carrier frequencies of the 12 *KIR* genes in the Ugandan population with frequencies in the UK cohort (Table 1). The Ugandan population showed marked differences in individual *KIR* gene frequencies; in particular, *KIR3DS1* and *KIR2DS1*, both associated with the *KIR* telomeric *B* region, were significantly less frequent. Additionally, comparatively higher frequencies of *KIR2DL2*, *KIR2DL5*, and *KIR2DS5* and lower frequencies of *KIR2DL3* and *KIR2DS3* were seen (*KIR2DS3* and *KIR2DS5* are alleles of the same gene). Increased *KIR2DL2* and decreased *KIR2DL3* frequency suggests that the centromeric *B* region is abundant in the Ugandan population. *KIR2DS5* can be found on either the centromeric or telomeric *B* regions in Africans, similarly to *KIR2DL5* in both European and African populations, making precise interpretation of centromeric *B* frequencies difficult (Hou et al. 2010). *KIR* gene frequencies in the Uganda population are similar to those reported for the South African Xhosa and San population (Middleton et al. 2007) indicating that African populations have lower frequencies of the telomeric *B* region and higher

frequencies of the centromeric *B* region compared with European populations.

We compared these results with data from a range of African populations where frequencies for *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR3DL1*, and *KIR3DS1* have been reported (Table 2). Of note is that the sample size was under 100 in 19 out of 22 studies. All African populations share similar characteristics compared to UK populations with lower *KIR2DS1* and *KIR3DS1* carrier frequencies; the highest value for *KIR2DS1* was 0.28 (Congo Mbuti) and 0.23 for *KIR3DS1* (Ethiopian) compared with, respectively, 0.42 and 0.44 for the UK population. The variability of these frequencies between the African populations may be visualized using principal component analysis (Fig. 1). The Ugandan population localizes to an intermediate position, between Central and West African populations and the East African populations. Therefore, in terms of *KIR* gene frequencies, the Ugandan population can be regarded as intermediate to these populations. The history of human migration in Africa is consistent with this observation because Uganda is an East African country known to have been populated from the Bantu migrations from West and Central Africa (Campbell and Tishkoff 2008). Furthermore, it is in both the northern and southern hemispheres, being situated on the equatorial line.

Previous reports have revealed extensive allelic diversity at individual *KIR* loci in African populations. To ensure all variants were detected, we used additional new primers that were added when positive results were obtained with only one pair of primers out of the two classically used or when the individual *KIR* gene content was unusual. Allele "dropout" was most commonly suspected for *KIR2DL3*, *KIR2DS1*, and *KIR2DS5*. This could be seen where the allele occurred in the

hemizygous or homozygous state and was detected in 2.4 % of the individuals. For *KIR2DS5*, this was usually observed on a contracted haplotype. Without using additional primers, published results for other African populations may not have detected these unusual alleles.

Analysis of the KIR2DL1 and KIR2DS1 ligand, *HLA-C* alleles encoding the C2 allotype, reveals that in general *C2* are more frequent than *C1* alleles in Africa compared with elsewhere in the world (Fig. 2). The Ugandan population, with a *C2* frequency of 54.9 % and a *C1* frequency of 45.1 % is
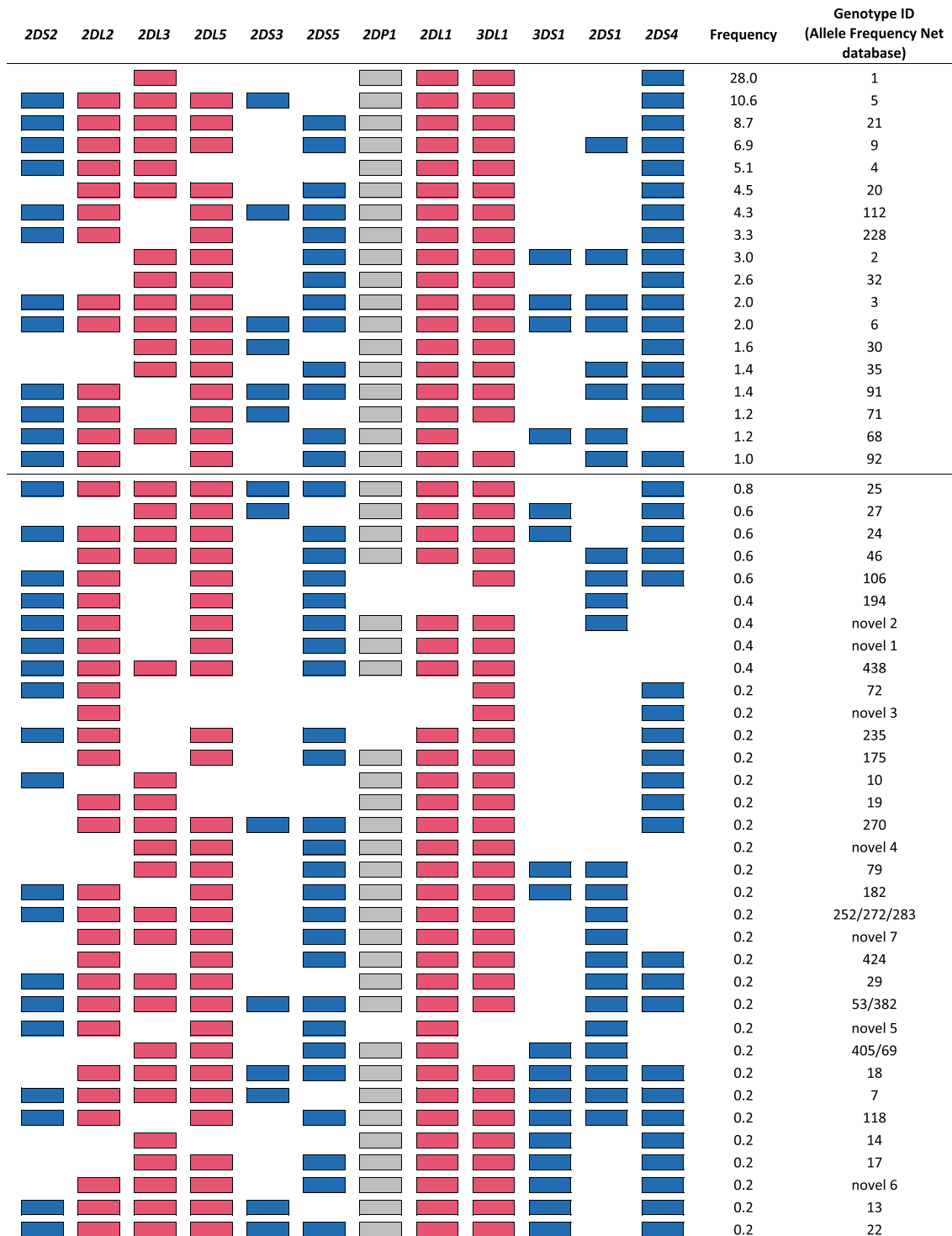

Fig. 3 *KIR* genotype profiles and frequencies observed in the Ugandan population. Reference numbers are according to the Allele Frequency Net database. New genotypes identified in this study are described as "novel."

Genes are presented in the order observed on sequenced KIR haplotypes except for *KIR2DS3S5* and *KIR2DL5*. Inhibitory genes are in *red*, activating genes are in *blue*, and the pseudo gene *KIR2DP1* is in *gray*

similar to the neighboring populations such as Kenyan Luo (*C2*=59 % and *C1*=41 %) or Rwandan (*C2*=55 % and *C1*=45 %) (Cao et al. 2004; Tang et al. 2000). The distribution of the *C2* group frequencies shows limited variation across the continent (Fig. 2). Therefore, in contrast to its activating receptor, *KIR2DS1*, African populations have high frequencies of *HLA-C2*, with comparatively little variation, in accordance with previous reports (Single et al. 2007). There must

Fig. 4 Inferred *KIR* haplotypes for 15 individuals representing 13 *KIR* genotypes. Genes are presented in the order observed on published sequenced *KIR* haplotypes. Inhibitory genes are in *red*, activating genes are in *blue*, and pseudo genes are in *gray*. Haplotype names are according to Pyo et al. (2013)
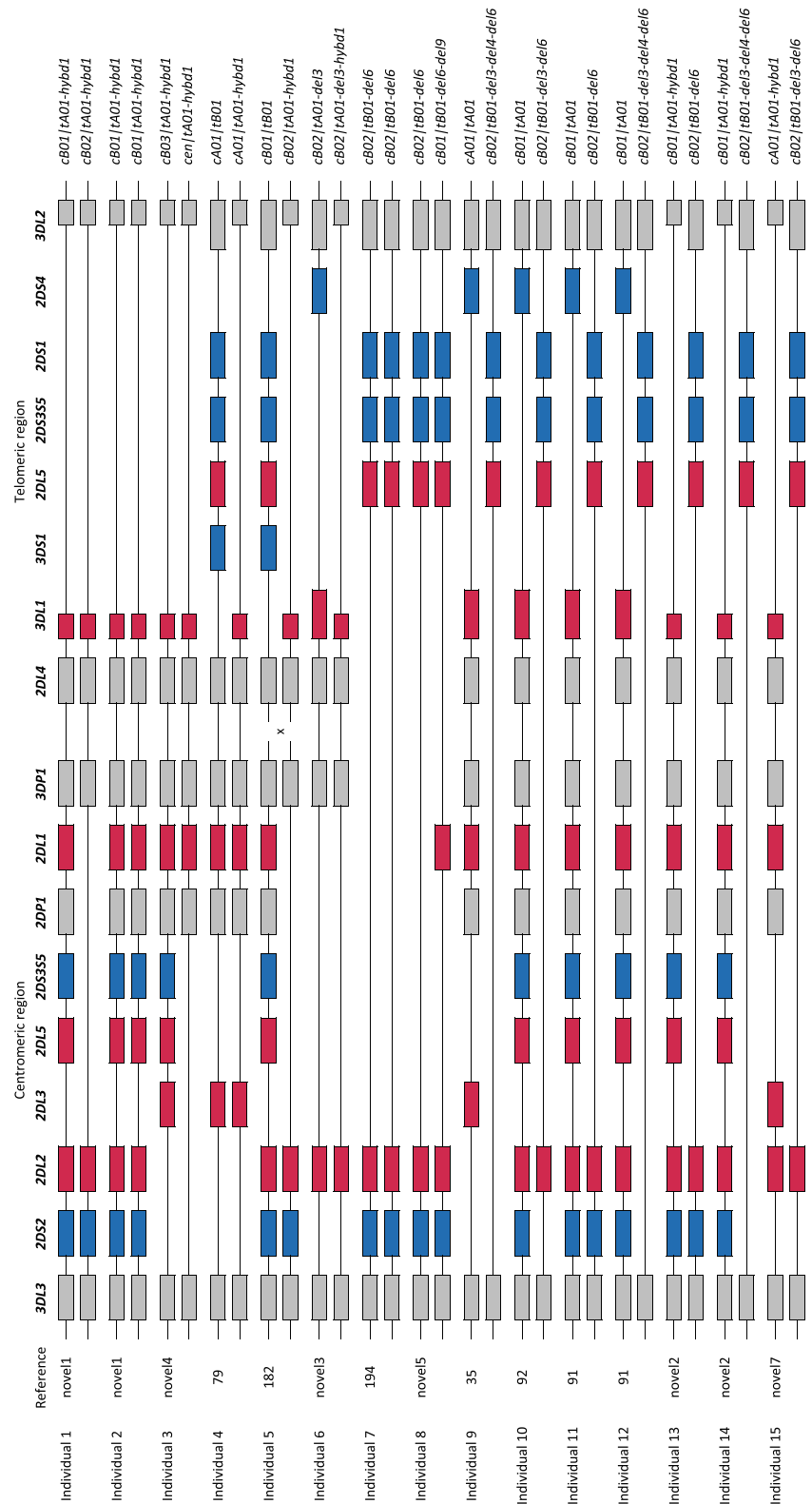
be strong selective pressures within Africa, either from pathogens or reproductive success that are maintaining this high level of C2.
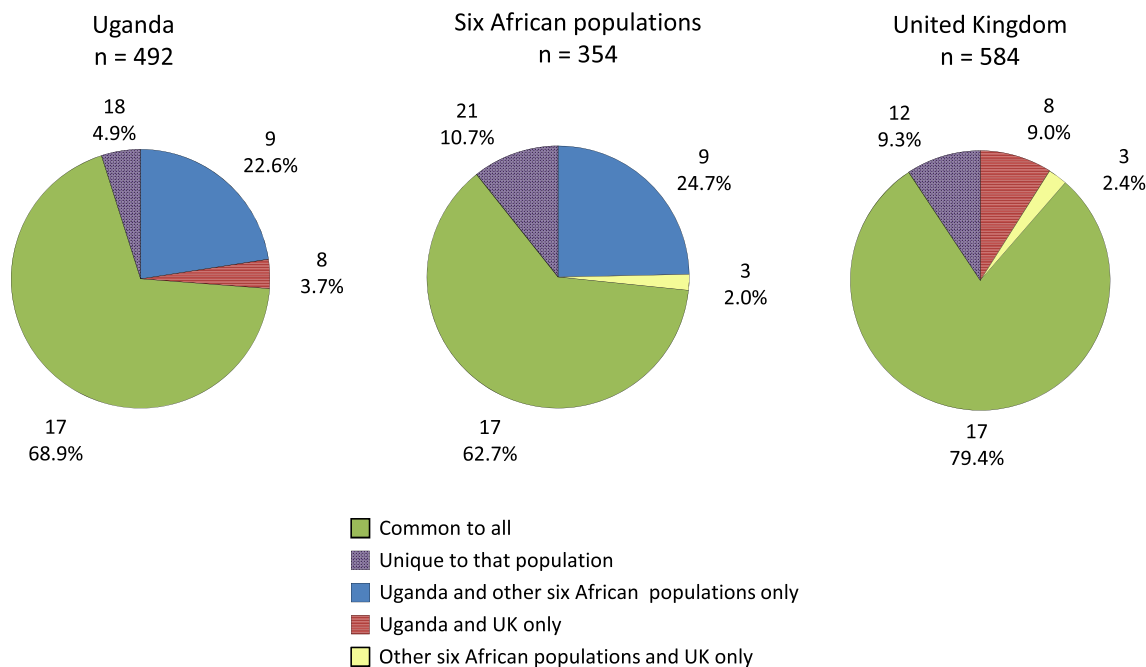
KIR genotypes

Genotyping of the entire cohort for the presence/absence of the 12 variable *KIR* genes allowed the definition of individual *KIR* genotypes. Fifty-two *KIR* gene content profiles were observed in the Ugandan population (Fig. 3). Nineteen genotypes had a frequency above 1 %, accounting for 90.0 % of individuals. Half of the individuals were classified as Ganda (both parents speaking the Ganda language, 50.9 %), but the other groups were broadly similar in *KIR* genotype frequencies (Table S 1). As in other African populations, the most frequent *KIR* genotype in the Ugandan population is homozygosity for the *KIR A* haplotype (28.1 %). The reported frequencies for this *KIR AA* genotype in Africa are variable, from 12 % in a South Africa Xhosa population to 42 % in Senegal (Williams et al. 2004; Yindom et al. 2010).

The remaining 33 genotypes are rare (<1 %), comprising 9.9 % of the total population. These represent unusual genotypes, including contracted or extended *KIR* haplotypes containing an unusual combination of *KIR* genes. In order to investigate these haplotypes, which arise from ancestral unequal recombination events, 15 individuals were fully typed for *KIR* gene copy number. Firstly, different regions of *KIR3DL1* (exons 4 and 9) and of *KIR3DL2* (exons 4 and 9) were separately typed for copy number to detect the *KIR3DL1/3DL2* hybrid gene (Shilling et al. 2002). Nine out of 15 individuals had one or two copies of this

telomeric region (tA01-hybd1; individuals 1–6 and 13–15, Fig. 4). Two additional genotypes (numbers 438 and 252/72/83, Fig. 3) have *KIR3DL1* present without *KIR2DS4*, and these individuals are also likely to have the same contracted telomeric region (*tA01-hybd1*). This contracted haplotype was observed in this Ugandan cohort at an underestimated frequency of 1.7 %, which is lower than the 6.5 % from other African populations (Norman et al. 2009; Norman et al. 2007), because the common telomeric *A* region (*tA01*) will hide this deletion, as seen with individual 6. Secondly, nine different individuals (individuals 7–15) have one or two copies of other contracted haplotypes defined by a deletion of the central framework genes, *KIR3DP1*, *KIR2DL4*, and *KIR3DS1*, defined as del6 (Pyo et al. 2010) and previously reported in individuals of African ancestry (Gómez-Lozano et al. 2003; Norman et al. 2002). Using these individuals and the similar *KIR* genotypes with *KIR2DS1* present without *KIR3DS1*, the frequency of this deletion in Uganda is greater than 7.25 %.
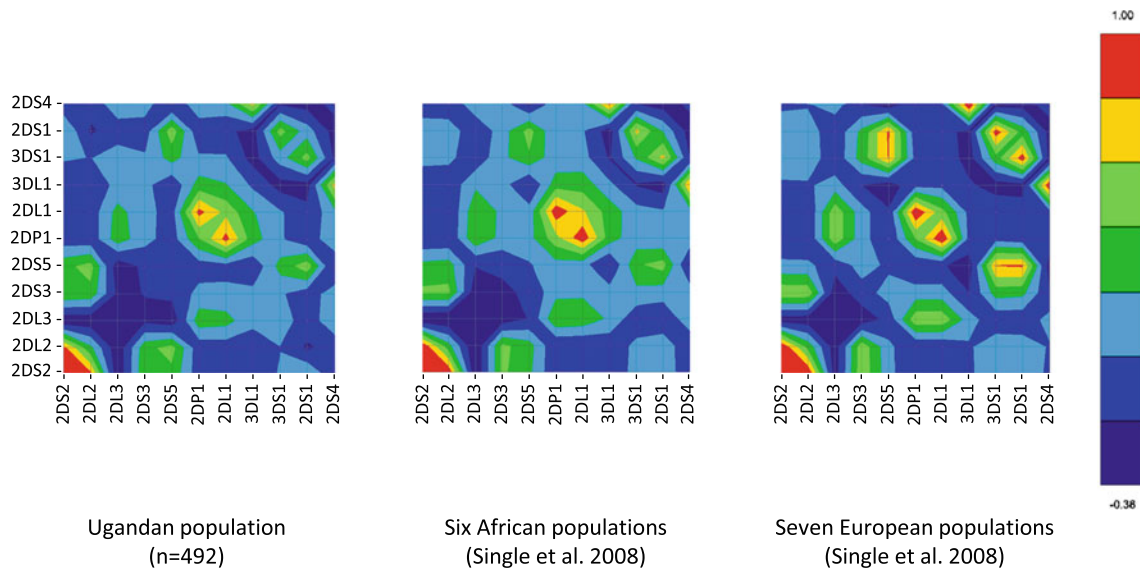
More *KIR* profiles were found in the Ugandan cohort (52 genotypes) when compared to a similar sized cohort of women from the UK (40 genotypes) (Fig. 5). The two populations only shared 25 genotypes present in 72.6 % of Ugandans and 88.3 % of UK. Similarly to the six other African populations (from the Allele Frequency Net database: Gabon, Ghana, Senegal, South Africa San, and South Africa Xhosa), the Ugandan population displays a higher diversity at the genotype level, and more than a quarter of these genotypes are not observed in Europeans. Forty-eight genotypes are unique to the African populations (including Uganda) and therefore might be African genotypes that have not spread out of Africa to Europe.

Fig. 5 Genotypes observed for the Ugandan population (*n*=492), six other African populations from the Allele Frequency Net database (Gabon, Ghana, Senegal, South Africa San, and South Africa Xhosa, *n*=354), and the UK population (*n*=584), showing proportions of common and unique genotypes for each group

Table 3 Linkage disequilibrium observed in the *KIR* region in the Ugandan population

	2DS2	2DL2	2DL3	2DL5	2DS3	2DS5	2DP1	2DL1	3DL1	3DS1	2DS1	2DS4
Wn*												
2DS2		0.867	−0.347	0.569	0.397	0.355	−0.095	−0.076	−0.104	0.033	0.224	−0.114
2DL2			−0.334	0.669	0.349	0.486	−0.110	−0.097	−0.087	0.011	−0.213	−0.109
2DL3				−0.278	−0.223	−0.320	0.330	0.290	0.063	0.116	−0.082	0.140
2DL5					0.412	0.733	−0.036	−0.016	−0.105	0.254	0.405	−0.154
2DS3						−0.130	0.077	0.068	0.081	0.044	−0.090	0.119
2DS5							−0.075	−0.051	−0.143	0.288	0.534	−0.210
2DP1								0.880	0.303	0.050	−0.139	0.196
2DL1									0.226	0.044	−0.135	0.144
3DL1										−0.260	−0.259	0.682
3DS1											0.495	−0.203
2DS1												−0.262
2DS4												
p value												
2DS2		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS	NS	0.005	NS	<0.0001	NS
2DL2			<0.0001	<0.0001	<0.0001	<0.0001	0.003	<0.0001	NS	NS	<0.0001	0.006
2DL3				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS	0.001	NS	0.002
2DL5					<0.0001	<0.0001	NS	NS	0.004	<0.0001	<0.0001	<0.0001
2DS3						0.001	NS	NS	NS	NS	NS	<0.0001
2DS5							NS	NS	<0.0001	<0.0001	<0.0001	<0.0001
2DP1								<0.0001	<0.0001	NS	<0.0001	0.001
2DL1									0.005	NS	<0.0001	0.001
3DL1										<0.0001	<0.0001	<0.0001
3DS1											<0.0001	<0.0001
2DS1												<0.0001
2DS4												

Ugandan population (n=492)
Six African populations (Single et al. 2008)
Seven European populations (Single et al. 2008)

Fig. 6 Comparison of the linkage disequilibrium observed in the *KIR* region between the Ugandan population and six African and seven European populations. The LD values calculated for 11 *KIR* gene loci for Ugandan population are compared with the values obtained using the same method by Single et al. (2008). *Red* represents strong positive LD, *blue* represents strong negative LD

Linkage disequilibrium

The LD between *KIR* genes was calculated for the 12 *KIR* loci in order to observe the pairwise association of genes in this population (Table 3). The strongest positive and negative LD values were observed between *KIR2DL1* and *KIR2DP1* (0.88) and *KIR2DL2* and *KIR2DL3* (−0.35), consistent with a previous report for six African populations (Single et al. 2008). A graphical analysis of Ugandan compared to these African populations and seven European populations is shown (Fig. 6). The positive LD between *KIR2DS1*, *KIR2DS5*, and *KIR3DS1* observed in Europeans is absent in Africans; in contrast, a positive association is seen between *KIR2DS5* and *KIR2DS2-KIR2DL2*, consistent with the location of *KIR2DS5* gene in the centromeric *B* region where *KIR2DS3* is generally located in Europeans. Overall, the LD values observed for the *KIR* genes are less extreme in Africa.

Conclusions

This Ugandan cohort is the largest African population typed for *KIR* genes, and when considered with other published data, the remarkable extent of *KIR* diversity in Africa is clear. The *KIR* and *HLA-C* frequencies observed in this population are consistent with other studies of African populations. A number of novel *KIR* gene profiles are described, including new genotypes and rare patterns. This East African population will be an important reference group for future disease and anthropological studies in African or African-derived populations. Our study has not considered the extensive allelic variation seen in Africans, for example, for *KIR3DL1* or *KIR2DL1*, where the great *KIR* diversity will become even more apparent.

Conflict of interest The authors have declared that no competing interests exist.

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