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KIR Variation in Iranians Combines High Haplotype and Allotype Diversity With an Abundance of Functional Inhibitory Receptors

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Natural killer (NK) cells are innate lymphocytes that eliminate infected and transformed cells. They discriminate healthy from diseased tissue through killer cell Ig-like receptor (KIR) recognition of HLA class I ligands. Directly impacting NK cell function, *KIR* polymorphism associates with infection control and multiple autoimmune and pregnancy syndromes. Here we analyze *KIR* diversity of 241 individuals from five groups of Iranians. These five populations represent Baloch, Kurd, and Lur, together comprising 15% of the ethnically diverse Iranian population. We identified 159 *KIR* alleles, including 11 not previously characterized. We also identified 170 centromeric and 94 telomeric haplotypes, and 15 different *KIR* haplotypes carrying either a deletion or duplication encompassing one or more complete *KIR* genes. As expected, comparing our data with those representing major worldwide populations revealed the greatest similarity between Iranians and Europeans. Despite this similarity we observed higher frequencies of *KIR3DL1*001* in Iran than any other population, and the highest frequency of HLA-B*51, a Bw4-containing allotype that acts as a strong educator of *KIR3DL1*001*$^+$ NK cells. Compared to Europeans, the Iranians we studied also have a reduced frequency of *3DL1*004*, which encodes an allotype that is not expressed at the NK cell surface. Concurrent with the resulting high frequency of strong viable interactions between inhibitory KIR and polymorphic HLA class I, the majority of *KIR-A* haplotypes characterized do not express a functional activating receptor. By contrast, the most frequent *KIR-B* haplotype in Iran expresses only one functional inhibitory KIR and the maximum number of activating KIR. This first complete, high-resolution, characterization of the *KIR* locus of Iranians will form a valuable reference for future clinical and population studies.

Keywords: NK cells, KIR, HLA class I, Iranian populations, immune diversity

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INTRODUCTION

Natural killer (NK) cells are essential for human immunity to infection and cancer, and for successful reproduction (1, 2). To discriminate diseased from healthy tissue cells, NK cells express an array of inhibiting and activating cell surface receptors (3, 4). Prominent among these receptors are the killer cell immunoglobulin like receptors (KIR), which educate and modulate NK cell function through interaction with HLA class I (5, 6). KIR are highly polymorphic, a genetically-determined variation that directly impacts NK cell function, and susceptibility to disease (7, 8).

The *KIR* locus spans 150–350 kbp of chromosome 19q13.4 (9). The locus is distinguished by structural and sequence diversity of the 13 constituent genes (*KIR2DL1*, *KIR2DL2/L3*, *KIR2DL4*, *KIR2DL5A*, *KIR2DL5B*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, *KIR3DL1/S1*, *KIR3DL2*, and *KIR3DL3*) and two pseudogenes (*KIR2DP1* and *KIR3DP1*) (10, 11). KIR have either two (2D) or three (3D) specificity-determining immunoglobulin-like domains and a long (L) or short (S) tail (12). KIR having a long cytoplasmic tail are inhibitory, whereas those having a short cytoplasmic tail are activating. The one exception is KIR2DL4, which can exhibit inhibitory or activating function (13–15). The *KIR* locus segregates in two main haplotype forms that are maintained in all human populations by balancing selection (16). The *KIR-A* haplotypes have a fixed number of predominantly inhibitory receptors, whereas the *KIR-B* haplotypes are characterized by a variable number of both activating and inhibitory receptors. Further distinguishing *KIR-A* and *-B* haplotypes are their characteristic alleles (17). *KIR-A* haplotypes are associated with controlling infectious disease and cancer, but confer susceptibility to reproductive disorders, whereas *KIR-B* haplotypes are associated with protection from reproductive disorders (1, 7). Additionally, haploidentical transplantation therapy for leukemia has an increased success rate when the stem cell donors carry *KIR-B* haplotypes (18, 19).

Polymorphism of *KIR* affects cell surface expression, ligand specificity, ligand binding strength, and intracellular signaling (20–25). All these factors affect the capacity of NK cells to recognize and kill target cells. Because both *KIR* polymorphism and associated diseases are unevenly distributed worldwide, it is critical to fully gauge the genetic diversity of *KIR* in well-defined human populations. Despite this importance, only a few populations have been studied to high resolution, principally due to the complexity of the *KIR* locus. These studies have focused on representative populations of Amerindians (Yucpa) (26), divergent African groups (27–29), Europeans (30, 31), East Asians (Japanese) (32), and Oceanians (Māori) (33). Together, they show how *KIR* allele and haplotype diversity varies dramatically between human populations and highlight the importance of extending *KIR* allele analysis to represent all ethnicities and geographical areas. In this regard, our recent analysis of *HLA* allelic diversity in Iran revealed the highest frequencies of *HLA-B*51:01* worldwide (34). HLA-B*51 contains the Bw4 epitope and interacts KIR3DL1 (35, 36). In the present study we fully characterize *KIR* locus diversity of the same cohort of Iranian individuals.

MATERIALS AND METHODS

Study Population

The *KIR* locus diversity of three indigenous Iranian populations was determined to high-resolution by analyzing genomic DNA from 241 healthy unrelated donors (34). The populations studied represent Baloch, Kurd, and Lur, together comprising 15% (12 million individuals) of the ethnically diverse Iranian population. Studied were 160 Lurs and 48 Kurds, from the Zagros Mountains at the west of Iran, and 33 Baloch from the southeast of Iran. The Lur population included 64 individuals from the city of Khoramabad in the province of Lorestan, 81 from Yasuj in the province of Kohgiluyeh and Boyer-Ahmad, and 15 from Lordegan in the province of Chaharmahal and Bakhtiari. The samples from Kurds were collected from the city of Sanandaj. With the exception of the Baloch, the *HLA class I* alleles were described previously (34). The ancestors of every individual studied had been part of their respective population for at least two generations. Sample collection was approved by the Medical Research Ethics Committee of Shiraz University of Medical Sciences. All participants gave informed consent. Banked, de-identified samples were used for this study.

Library Preparation and Enrichment

Genomic DNA was prepared by shearing with sonication and the *KIR* genomic region was enriched from the genomic libraries using a pool of oligonucleotide probes as described (37). The enriched fragments were subjected to paired-end sequencing using Illumina's MiSeq instrument and V3 sequencing chemistry (Illumina, La Jolla CA). The sequencing read length was 2 × 300 bp.

Next Generation Sequence Data Processing and Analysis

Sequence reads specific to the *KIR* region were identified and harvested using Bowtie 2 (38). KIR genotyping was performed using the Pushing Immunogenetics to the Next Generation pipeline (37). This pipeline generates a high-resolution *KIR* gene content and allele level genotype. It can also identify previously unreported single nucleotide polymorphisms (SNPs) and recombinant alleles. Novel allele sequences were analyzed by visual inspection: reads specific to the relevant gene were isolated by bioinformatics filtering, aligned to the closest reference allele using MIRA 4.0.2 (39), and inspected using Gap4 of the Staden package (40) or Integrative Genomics Viewer (41).

Allele and Haplotype Frequencies

Allele frequencies were calculated by direct counting. The composition and frequencies of *KIR* haplotypes were determined at the allelic level using PHASE 2.1 (42). The following parameters for PHASE 2.1 were used: –f1, –x5, and –d1. Because of the high rate of recombination between *KIR3DP1* and *KIR2DL4* (9), we performed two separate PHASE runs, one for the *KIR* genes of the centromeric region and one for telomeric *KIR* genes. Genes analyzed were *KIR3DL3, 2DS2, 2DL2/3, 2DL5A* and *B, 2DS3/5, 2DP1, 2DL1, 2DL4, 3DL1/S1,*

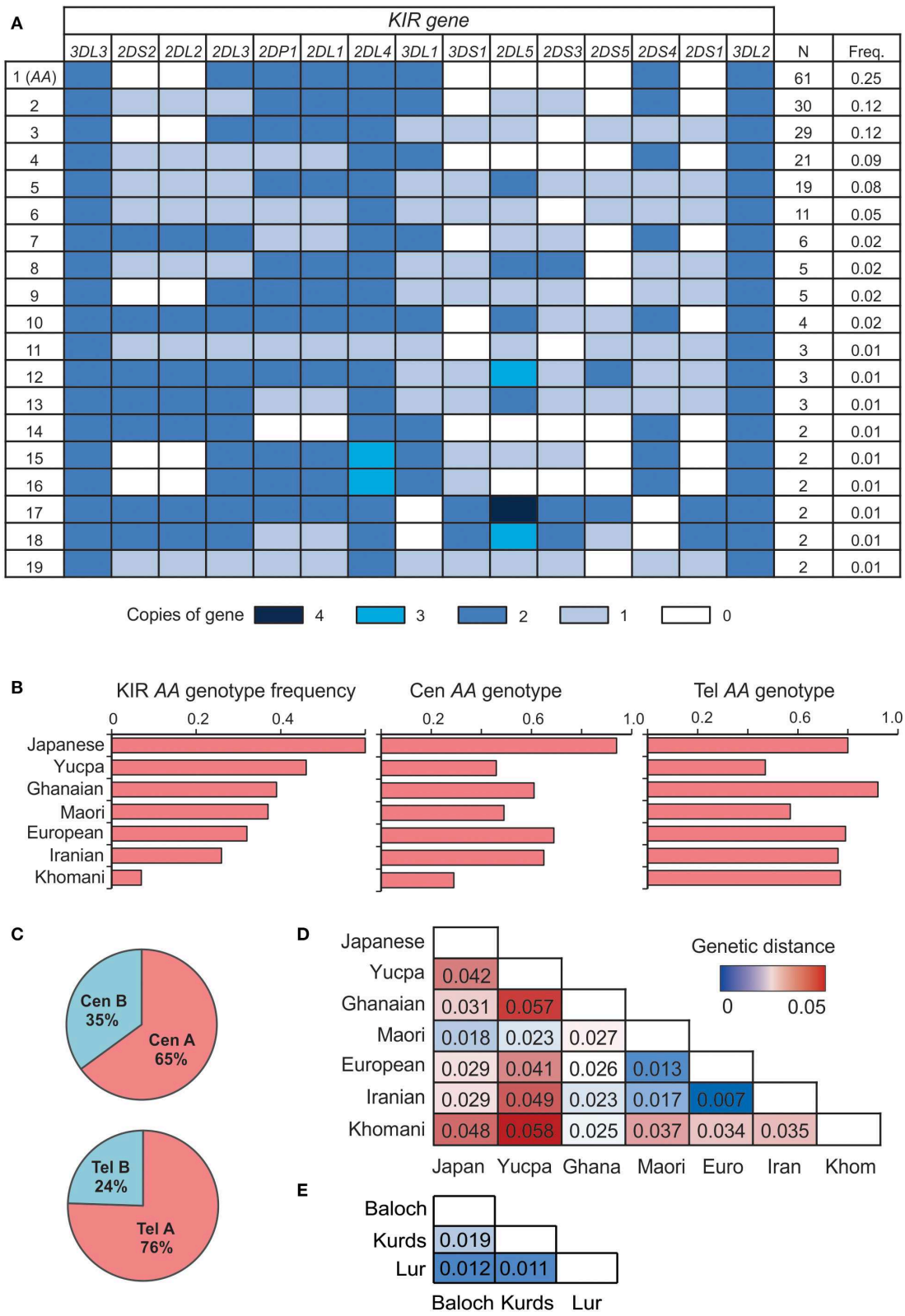


FIGURE 1 | Iranian *KIR* genotypes resemble those of Europeans. **(A)** Shows *KIR* gene copy-number genotypes ordered by the total number observed across the five Iranian populations. Only genotypes present in more than one individual are shown. Colored boxes indicate the number of copies of the gene, as given in the key

(Continued)

FIGURE 1 | below. A white box indicates the gene is absent. **(B)** Frequency of the full *KIR AA* (left), *Cen AA* (center), and *Tel AA* (right) genotypes across seven representative world populations, ordered by frequency of full *KIR AA*. The populations are Japanese ($N = 115$), Yucpa Amerindians ($N = 61$), Ghanaians from West Africa ($N = 131$), Maori from New Zealand ($N = 49$), Europeans ($N = 378$), Iranians ($N = 241$), and Khomani from South Africa ($N = 79$). **(C)** Frequency of centromeric (Cen) and telomeric (Tel) *KIR A* and *B* haplotypes in the five Iranian populations combined. **(D)** Genetic distance between the combined Iranian population and the six representative, populations from **(B)**. *KIR* genes included are those genotyped in all the populations shown *2DL1-4, 2DS1-5, 3DL1/S1, 3DL2*. **(E)** Genetic distance between the three Iranian populations.

2DS1, 2DS4, and *3DL2*. For each haplotype we calculated the frequency by direct counting.

Statistical Analysis

Hardy-Weinberg equilibrium proportions was examined using Fisher's exact test. Differences in frequency amongst populations were tested using χ^2 with Bonferroni correction for the number of alleles at the respective locus. Fisher's and χ^2 test as well as Mann-Whitney *U*-test were implemented using GraphPad Prism 7.05.

Genetic Distance

The genetic distance between Iranians and other populations was calculated using the Cavalli-Sforza model (43), implemented in GENETIX 4.05 (https://kimura.univ-montp2.fr/genetix/). The populations used were Japanese ($N = 115$) (32), Yucpa Amerindians ($N = 61$) (26), Ghanaians from West Africa ($N = 131$) (27), Māori from New Zealand ($N = 49$) (33), Europeans ($N = 378$) (44), and Khomani from South Africa ($N = 79$) (28).

RESULTS

We sequenced the *KIR* genes of 241 individuals from five populations, representing three groups of Iranians; the Lurs, Kurds and Baloch. The Lurs comprised individuals from the cities of Khoramabad, Lordegan and Yasuj. Only two *KIR* genes were present as two copies (2N) in every individual, *KIR3DL3* at the centromeric end of the *KIR* locus and *KIR3DL2* at the telomeric end (**Figure 1A**). Every *KIR* haplotype in this Iranian cohort is therefore flanked by these two framework genes. The third framework gene is *KIR2DL4* (9). Because eight individuals have only one copy of *KIR2DL4* and six have three copies, *KIR2DL4* is not present on every Iranian *KIR* haplotype (**Figure 1A**), but is likely duplicated on some haplotypes and deleted from others (45). The frequency of *KIR-A* haplotypes varies across populations (16, 46). In the combined study population, the frequency of *KIR AA* genotypes observed is 25% (**Figure 1B**). C*entromeric KIR-A* haplotypes are present at 65% and *telomeric KIR-A* haplotypes at 76% (**Figure 1C**). The frequency of *KIR A* haplotype homozygotes closely matches that of European populations (**Figure 1B**), as do genetic distance measurements calculated from the *KIR* genotype data (**Figure 1D**). In conclusion, the number and distribution of *KIR* gene content haplotypes in Iranians closely resemble those present in Europeans.

We determined the *KIR* allele frequencies in the five Iranian populations. These data are shown in **Supplementary Material A** and summarized in **Figure 2**. The allele frequencies were consistent with Hardy-Weinberg equilibrium. Among the five populations, we identified 115 inhibitory KIR alleles and 18 activating KIR alleles (**Figure 2A**). Also present are 12 *KIR2DL4* alleles. Inhibitory KIR are highly polymorphic in Iranians, with 12 *KIR2DL1*, 9 *KIR2DL2/L3*, 10 *KIR2DL5*, 22 *KIR3DL2*, 18 *KIR3DL1*, and 44 *KIR3DL3* alleles observed in total. As in other populations, the activating KIR are less polymorphic than the inhibitory KIR, with 7 *KIR2DS4* alleles, 8 *KIR2DS3/5* alleles, two *KIR2DS1* and two *KIR2DS2* alleles and one *KIR3DS1* allele being seen (**Figure 2A**). In Iranians, *KIR3DL3* is the most polymorphic *KIR* gene, whereas *KIR2DS1* and *KIR2DS2* are the least variable. In the combined population analyzed, the most frequent allele for each of the inhibitory KIR are *2DL1*00302*, *2DL2*00101*, *2DL3*00101*, *3DL1*00101*, *3DL2*00101*, and *3DL3*00301,* respectively (**Supplementary Material A**). The most common KIR2DL4 and KIR2DS4 alleles are *2DL4*00801* and *2DS4*003*, respectively. For the other activating KIR (KIR2DS1-3 and 2DS5) as well as KIR2DL5, the most frequent allele observed is absence of the gene (**Supplementary Material A**). We did not observe any statistically significant difference in frequency of any specific *KIR* allele between Kurs and Lurs. We identified 11 novel *KIR* alleles, eight being defined by amino acid substitutions, one by a synonymous substitution and two by substitutions in *KIR2DP1* pseudogene (**Figure 2B**). All these novel alleles were observed in one or two individuals (**Figure 2B**). Seven of them have sequences identical to ones reported recently in a survey of more than one million registered bone marrow donors (47).

On the basis of allele composition, we defined 170 centromeric and 94 telomeric *KIR* haplotypes (**Supplementary Materials B,C**). Thus, by distinct haplotype number, the centromeric region is twice as diverse as the telomeric region. Emphasizing this difference, the 55 most frequent centromeric haplotypes account for 75% of the total haplotypes, whereas only 13 telomeric haplotypes are sufficient to account for 75% of the telomeric haplotypes (**Figures 3A,B**). In conclusion, the centromeric *KIR* region of Iranian *KIR* haplotypes is far more diverse than the telomeric *KIR* region.

Although *KIR-A* haplotypes are more numerous and frequent than *KIR-B* haplotypes (**Figure 1**), the most frequent centromeric region and the second most frequent telomeric region haplotypes are *KIR-B* (**Figures 3C,D**). Together, these centromeric and telomeric segments encode only one inhibitory receptor specific for HLA class I (KIR2DL2*001). Also encoded are KIR2DL1*004, an attenuated receptor (23) and KIR3DL2*007, which has a mutation in the first ITIM of the cytoplasmic tail (48) and thus may not transmit an inhibitory signal. By contrast, the *KIR-B* haplotype encodes three functional activating receptors (KIR2DS1, 2DS2, and 3DS1) and a full-length KIR2DL4 (*005). This combination of common centromeric and telomeric *KIR-B*

A

	Baloch (N=33)		Kurds (N=48)		Lurs						
					Lorestan (N=64)		Lordegan (N=15)		Yasuj (N=81)		
KIR gene	k	H	k	H	k	H	k	H	k	H	Total
2DL1	7	0.94	8	0.81	8	0.85	7	0.86	9	0.68	12
2DL2/3	5	0.76	6	0.67	5	0.75	5	0.73	8	0.76	9
2DL4	8	0.79	8	0.81	8	0.82	8	0.93	10	0.82	12
2DL5	7	0.63	6	0.48	6	0.51	3	0.53	6	0.73	10
2DS1	1	0.42	1	0.33	1	0.31	1	0.4	2	0.43	2
2DS2	1	0.36	1	0.44	2	0.46	1	0.73	1	0.48	2
2DS3/5	3	0.64	3	0.60	4	0.57	3	0.53	5	0.70	7
2DS4	5	0.79	5	0.62	5	0.80	5	0.8	6	0.76	6
3DL1/S1	10	0.79	15	0.83	14	0.86	9	0.93	15	0.85	19
3DL2	10	0.79	16	1.00	17	0.87	9	0.93	17	0.81	22
3DL3	20	0.94	26	0.96	26	0.97	15	0.93	39	0.99	44

B

KIR gene	Closest allele	Nucleotide change	Coding change	Domain	N
2DL1	**00201*	G 331 T †	V 111 L	D0	1
	**00302*	G 421 A †	A 141 T	D1	2
2DL4	**00801*	G 568 A †	G 190 R	D2	2
	**00801*	C 883 A	P 295 T	Cyt	1
3DL3	**01402*	G 102 A †	W 34 X	D0	1
2DS4	**003*	A 110 C †	H 37 P	D1	2
2DS5	**00201*	C 252 T †	syn	D0	1
3DS1	**01301*	G 775 C	G 259 R	D2	1
	**01301*	T 1160 C	F 387 L	Cyt	2
2DP1	**00301*	C 702 A †	-	-	
		C 703 G †	-	-	2
	**00201*	C 1107 T	-	-	1

FIGURE 2 | High *KIR* diversity and allele discovery in Iranians. **(A)** Shows the numbers of *KIR* alleles (k) and the heterozygosity (H) in each of the five Iranian populations. The total number observed is shown at the right. Gene absence is not included as an allele. **(B)** Shows the novel *KIR* alleles identified in this study. Columns from left to right are: the *KIR* gene, the closest known allele, the nucleotide change compared to the closest allele, the amino acid substitution caused by the nucleotide change, domain affected by the amino acid substitution (LP, leader peptide; D0–D1, Ig-like domains; TM, transmembrane domain) and the number observed. † -indicates identical allele observed by Wagner et al. (47).

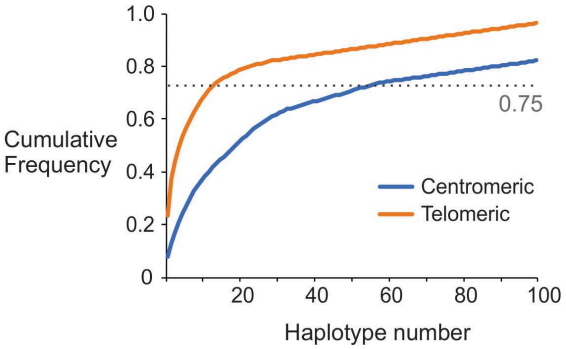
haplotypes can therefore provide the maximum number of activating KIR. The most frequent *KIR-A* haplotype encodes four inhibitory receptors specific for polymorphic HLA class I and carries *KIR2DS4*003*, which is not-expressed because of a 22 bp deletion in exon 5 (11). Indeed, the frequency of the *KIR2DS4 22 bp-del* variant in Iranians is 0.62, more than four times higher than the frequency of the full-length variant (**Figure 3E**). Consequently, almost all *KIR-A* haplotypes in Iranians encode four functional inhibitory receptors and no activating receptor specific for polymorphic HLA class I.

A characteristic of the *KIR* locus is the occurrence of large-scale duplication or deletion events that encompass complete genes, which synergizes with allele variation to enhance KIR functional diversity (45, 49, 50). In the Iranian cohort, we identified seven *KIR* haplotypes having large deletions and eight with duplications (**Figure 4**). One of these haplotypes (number 1 in **Figure 4**) was not observed previously and is similar to the most frequent *KIR-B* haplotype in Iran, with the difference being that it lacks *KIR2DS1*. This haplotype could have been formed by homologous or looping out recombination (50). The remaining

A

KIR haplotypes	Number (Distinct) A	B
Centromeric	112	59
Telomeric	66	34

B



C

	Centromeric *KIR* Haplotypes								
	3DL3	*2DS2*	*2DL2/3*	*2DL5*	*2DS3/5*	*2DP1*	*2DL1*	N	Freq.
1	*00301	*00101	2*00101	B*00201	3*00103	*00102	*00401	40	0.166
2	*00101		3*00101			*00201	*00302	25	0.104
3	*00901		3*00101			*00201	*00302	22	0.091
4	*00902		3*00101			*00201	*00302	17	0.071
5	*01501		3*00101			*00201	*00302	17	0.071
6	*01101		3*00101			*005	*00302	15	0.062
7	*007	*00101	2*00301					13	0.054
8	*02701		3*00501			*016	*001	13	0.054

D

	Telomeric *KIR* Haplotypes								
	2DL4	*3DL1/S1*	*2DL5A*	*2DS3/5*	*2DS4*	*2DS1*	*3DL2*	N	Freq.
1	*00801	*00101			*003		*00101	116	0.481
2	*00501	S1*01301	A*00101	S5*00201		*00201	*00701	71	0.295
3	*00103	*008			*003		*00901	39	0.162
4	*00102	*002			*00101		*00201	26	0.108
5	*00602	*00701			*004		*008	23	0.095
6	*011	*00501			*010		*010	17	0.071
7	*00102	*01502			*00101		*00201	15	0.062
8	*00802	*00401			*006		*00501	14	0.058
9	*00501	S1*01301	A*00501	S3*00201		*00201	*00701	13	0.054
10	*00802	*00402			*006		*00501	12	0.050

E

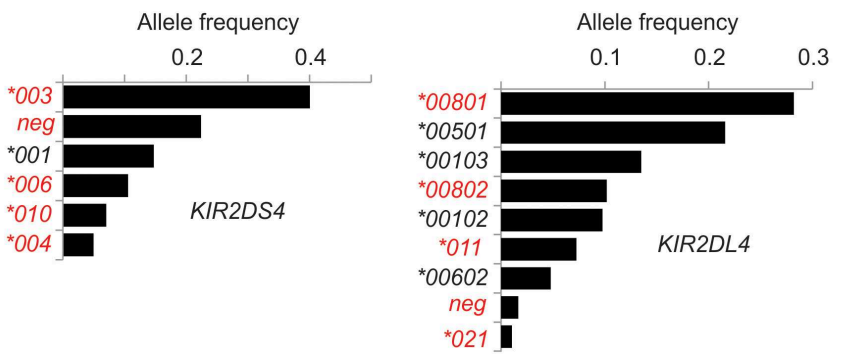


FIGURE 3 | Centromeric *KIR* are more diverse than telomeric *KIR* in Iranians. **(A)** Shows the number of distinct haplotypes observed in the centromeric and telomeric *KIR* regions. **(B)** Shows the cumulative frequency of haplotypes observed in the centromeric and telomeric *KIR* regions. **(C,D)** Shows the allele composition of all **(C)** centromeric, and **(D)** telomeric *KIR* haplotypes identified in more than ten individuals in the combined Iranian population. *KIR A* haplotypes are shaded pink and *KIR B* haplotypes are blue. Empty boxes indicate gene absence. At the right is shown number observed and the frequency in the combined Iranian population ($N = 241$). All the observed haplotypes are given in **Supplementary Material (E)**. Shown are the frequencies of *KIR2DL4* and *KIR2DS4* alleles in the combined Iranian population. Red text indicates alleles not expressed at cell surface. The allele frequencies for all *KIR* genes in each of the five populations are given in **Supplementary Material**.

	KIR gene															
	3DL3	*2DS2*	*2DL2/3*	*2DL5*	*2DS3/5*	*2DP1*	*2DL1*	*2DL4*	*3DL1/S1*	*2DL4*	*3DL1/S1*	*2DL5*	*2DS3/5*	*2DS1*	*2DS4*	*3DL2*	N
1	**01309*		*3*00201*			**00301*	**00302*			**00501*	*S1*01301*	*A*00101*	*5*00201*			**00701*	1
2	**036*	**001*	*2*00101*	*B*00801*	*5*00201*									**00201*		**00701*	1
3	**00301*	**001*	*2*00101*	*B*00801*	*5*00201*									**00201*		**00701*	2
4	**00301*	**001*	*2*00301*	*A*00102*	*5*00201*									**00201*		**00701*	1
5	**00301*	**001*	*2*00101*	*B*00201*	*3*00201*									**00201*		**019*	1
6	**00202*	**001*	*2*00301*	*B*00201*	*3*00201*									**00201*		**00701*	1
7	**00301*	**001*	*2*00101*													**00701*	1
8	**00301*	**001*	*2*00101*	*A*00501*	*3*00103*	**00102*	**00401*	**00501*	*S1*01301*	**00801*	*L1*00101*				**00101*	**00202*	1
9	**00202*		*3*00101*			**005*	**00302*	**00501*	*S1*01301*	**00103*	*L1*008*				**003*	**00701*	1
10	**00901*		*3*00101*			**00201*	**00302*	**00501*	*S1*01301*	**00801*	*L1*00101*				**003*	**00101*	1
11	**00202*		*3*00101*			**00201*	**00302*	**00501*	*S1*01301*	**00102*	*L1*01502*				**00101*	**00201*	1
12	**00301*	**001*	*2*00101*	*B*00201*	*3*00201*	**00102*	**00401*	**00801*	*L1*00101*	**00501*	*S1*01301*	*A*00501*	*5*00201*		**003*	**00701*	1
13	**041*		*3*00501*			**00102*	**00401*	**00801*	*L1*00101*	**00501*	*S1*01301*	*A*00501*	*3*00103*		**003*	**00901*	1
14	**00101*	**001*	*2*00101*	*B*00201*	*3*00103*	**00102*	**00401*	**00801*	*L1*00101*	**00501*	*S1*01301*	*A*00501*	*3*00103*		**003*	**00101*	1
15	**00301*	**001*	*2*00101*			**00301*	**010*	**00801*	*L1*00101*	**00501*	*S1*01301*	*A*00501*	*3*003N*	**00201*	**00101*	**00701*	1

Deleted segment Duplicated segment

FIGURE 4 | Rare structural variants of KIR haplotypes in Iranians. Shown are Iranian *KIR* haplotypes affected by a copy number variation. Gray and purple boxes highlight deleted or duplicated segments, respectively. "*N*" indicates the number observed.

deletion haplotypes are similar to those observed worldwide including Africans, and the duplication haplotypes similar to those observed outside of Africa (45).

The interaction of HLA class I with inhibitory KIR contributes to NK cell education (51, 52). We analyzed the distribution of alleles for the four inhibitory KIR that are specific for HLA class I in Iranians and compared them with six other populations that represent the breadth of human genetic diversity (**Figure 5**). This analysis showed that in Iranians, Europeans, West Africans, and Japanese the most frequent *KIR2DL1* and *KIR2DL2/3* alleles are *2DL1*003* and *2DL3*001*, respectively. The two populations that differ are relatively small indigenous populations (**Figures 5A,B**). By contrast, the *KIR3DL1/S1* and *KIR3DL2* alleles most frequent in Iranians are usually not the same as those most frequent in other populations (**Figures 5C,D**). This shows there is a greater worldwide divergence of KIR specific for HLA-A and -B than of KIR specific for HLA-C. Of particular note, Iranian populations have the highest frequency of *3DL1*001* (0.29) compared to the other six populations as well as to all other populations analyzed to date (53). Allele frequencies of the four inhibitory *KIR* specific for HLA class I are similar across the Iranian populations analyzed (**Figures 5E–H**). The one exception is the Baloch who have a low frequency of *KIR3DL1*004*, as well as *KIR3DL2*003* and **005,* which are in strong linkage disequilibrium with *3DL1*004* (28, 44) (**Supplementary Material C**). The Baloch have one tenth the frequency of *3DL1*004* (0.015) than Kurd [0.114, $\chi 2$ $p = 0.006$; pc = 0.06(ns)], Lorestan [0.109, $\chi 2$ $p = 0.006$; pc = 0.06(ns)] and Yasuj [0.129, $\chi 2$ $p = 0.003$; pc = 0.03]. KIR3DL1*004 is retained in the cytoplasm and unable to bind HLA-Bw4+ HLA-A or -B on target cells (54).

We examined the compound genotypes of *KIR* and *HLA class I*, to determine the potential number of interactions between HLA class I ligands and inhibitory KIR. We observed a consistent mean of four viable interactions per individual of HLA-C with inhibitory KIR across the five Iranian populations (**Figure 6A**). This number is similar to the mean of 3.6 observed in Europeans (28). Although we observed no significant difference in the potential interactions of KIR3DL1 with HLA-A, the Baloch and Lordegan have more interactions of KIR3DL1 with HLA-B than the other three Iranian populations (**Figures 6B,C**). Despite the small numbers of individuals in these groups, the differences are statistically significant (Mann-Whitney *U*-test; $p < 0.013$). Contributing to these differences is the high frequency of HLA-B*51 in the Baloch (0.29; **Supplementary Material D**). This frequency is similar to the 0.28 observed in the Lordegan and considered the highest worldwide (34).

DISCUSSION

This study applied high-throughput, next-generation sequencing to define *KIR* polymorphism at high resolution in five Iranian populations. These comprise the Kurd and Lur populations from the Zagros Mountains in the west of Iran and the Baloch from the south-east. The Lur comprised three subpopulations; the Lorestan, Lordegan, and Yasuj. *HLA class I* allele distributions for four of these populations were reported previously (34) whereas those for the Baloch are described here. When we compared the *KIR* allele frequencies of Iranians to those representing African, Asian, European, Oceanian, and South American populations, we found that the Iranian groups we studied are particularly similar to Europeans. This finding is consistent with ancient DNA analysis, which revealed that Iranians and Europeans both originate from an Indo-Europeans steppe ancestor population (55).

Despite their overall similarity, the Iranians we studied differ from Europeans in their frequencies of *KIR3DL1*001*, a high-expressing inhibitory receptor specific for the Bw4 epitope of

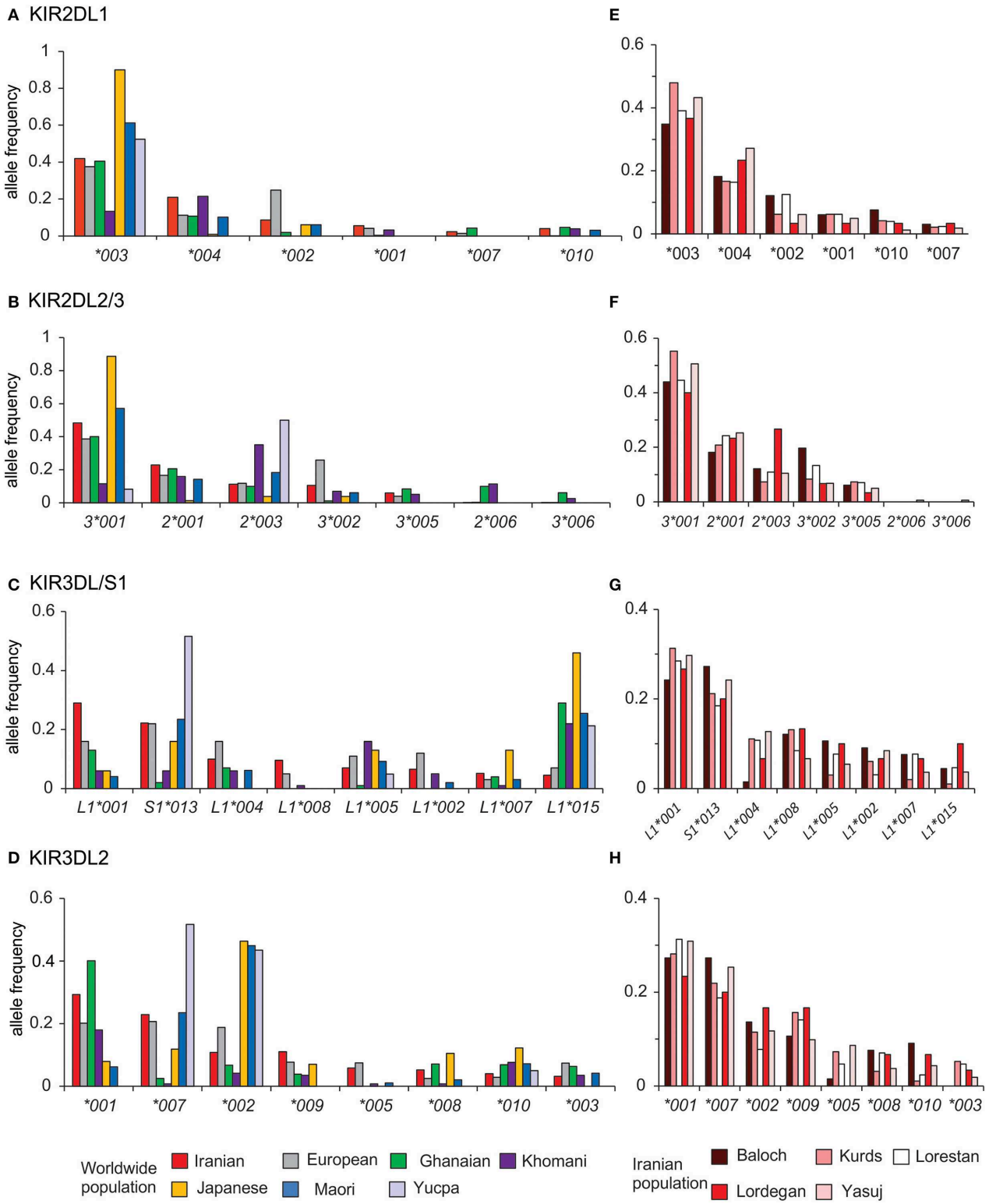


FIGURE 5 | Telomeric *KIR* are more divergent than centromeric *KIR* across populations. Shows the alleles of **(A)** *KIR2DL1*, **(B)** *KIR2DL2*/3, **(C)** *KIR3DL1/S1*, and **(D)** *KIR3DL2* observed in Iran, and their frequencies in the combined Iranian population and six other populations representing major world groups (Left). **(E-H)** show the allele frequencies in the individual Iranian populations. The populations are Iranians ($N = 241$, comprised from Baloch $N = 33$, Kurds $N = 48$, Lorestan $N = 64$, Lordegan $N = 15$, and Yasuj $N = 81$), Ghanaians ($N = 131$), Khomani ($N = 79$), Maori ($N = 49$), Japanese ($N = 115$), Europeans ($N = 378$), and Yucpa ($N = 61$). The allele frequencies for all *KIR* genes in each of the five Iranian populations are given in **Supplementary Material**.

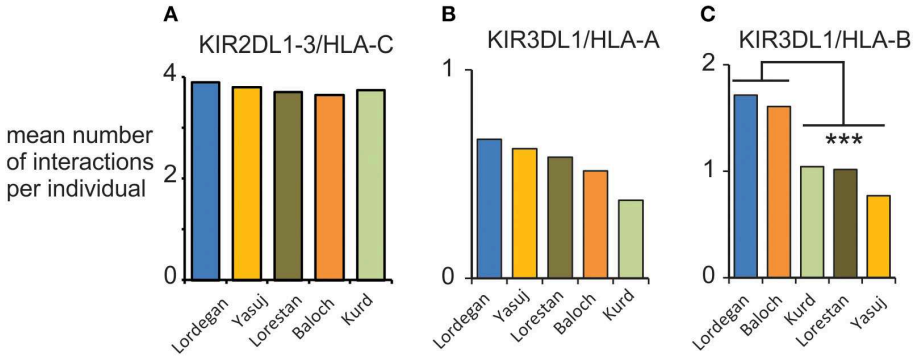


FIGURE 6 | Interactions between KIR and HLA class I in five Iranian populations. **(A)** Shows the mean total number of viable interactions per individual of inhibitory KIR and HLA-C. **(B)** Shows the mean total number of viable interactions per individual of KIR3DL1 and HLA-A. **(C)** Shows the mean total number of viable interactions per individual of KIR3DL1 and HLA-B. ***$p < 0.013$, obtained from a two-tailed Mann-Whitney *U*-test.

subsets of HLA-A and -B allotypes. Iranians have *KIR3DL1*001* frequencies that are twice those in Europeans and are the highest worldwide. Iran has the highest frequency of *HLA-B*51* in the world (34), and this is also the case for the Baloch. HLA-B*51 has the Bw4 epitope and thus educates KIR3DL1$^+$ NK cells to detect any loss in Bw4$^+$ HLA-A or -B expression (35, 56, 57). HLA-B*51 is associated with Behçet disease, a chronic, multi-system autoimmune condition that has substantially higher incidence in Iran (80/100,000) than Europe (<1/100,000) (58, 59). We recently showed that high-expressing allotypes of KIR3DL1, including 3DL1*001, can protect from Behçet disease (60). It is unlikely that KIR3DL1*001 and HLA-B*51 rose to high frequency in Iran to protect specifically from an autoimmune disease, but this combination of HLA and KIR could also protect against specific infectious diseases (7). Examples include tuberculosis and hepatitis, which are both prevalent in Balochistan (61–63). Amongst Iranians, the Baloch and Lordegan have the highest number of viable interactions between KIR3DL1 and Bw4$^+$HLA. Contributing to this high occurrence in the Baloch, are high frequencies of KIR3DL1*001 and HLA-B*51 and a low frequency of KIR3DL1*004. That both the *KIR3DL2* alleles linked to *KIR3DL1*004* are also reduced in frequency suggests that KIR3DL1*004 has been specifically targeted by negative selection in the Baloch, rather than another *KIR* allele in linkage disequilibrium with *KIR3DL1*004*.

KIR-A and *-B* haplotypes are present in all human populations, where they are maintained by balancing selection, likely because *KIR-A* haplotypes favor infection control, particularly viral infections, and *KIR-B* haplotypes favor successful fetal implantation (1, 16). Accordingly, *KIR-A* haplotypes express all possible inhibitory receptors specific for HLA class I, whereas *KIR-B* haplotypes express fewer inhibitory receptors but more activating receptors. Thus, whereas the inhibitory KIR help prime NK cells to be able to be responsive to HLA class I loss during infection, the activating KIR can both promote fetal trophoblast invasion and recognize specific pathogen-derived peptides to control certain infections (64, 65). In Iranian populations the differences between the *KIR-A* and *KIR-B* haplotypes is extreme. The common *KIR-A* haplotypes express no activating KIR, due to the high frequency of the truncated KIR2DS4 variant, and the common *KIR-B* haplotypes provide the maximum number of activating receptors. In this regard, the *KIR-B* haplotypes differ considerably from those of Africans and were likely obtained since the out of Africa migration, through adaptive introgression with ancient humans (66).

In summary, we describe the *KIR* locus at allelic resolution in Iranian populations and place it in the context of the HLA ligands recognized by KIR. Iran is a culturally diverse country and the ethnic groups we have studied comprise ∼15% of the population (67). Because substantial *KIR* gene content diversity is observed across Iran (68–71), it will be of interest in future studies to compare our results with other Iranian populations including Persians and Azeris. The allele and haplotype distributions described here will provide a baseline for future studies of disease association and transplantation matching in this important region of the world.

DATA AVAILABILITY STATEMENT

All relevant data are included in the article/**Supplementary Material**. Other raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Research Ethics Committee of Shiraz University of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PP, EA, AG, and PN conceived and designed experiments. EA, NN-G, and ST performed lab experiments. CA, PN, JAT, LG, NN-G, and ST analyzed data. AG, PP, WM, JH, JT, and LM provided materials. CA, PN, and PP wrote the paper. All authors approved the final submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.00556/full#supplementary-material

Supplementary Material (Spreadsheet) | (A) Shown are the allele frequencies for the 13 *KIR* genes in five Iranian populations, and the combined frequency is shown at the right. **(B)** Shown are the *centromeric KIR* haplotypes identified in five Iranian populations. The combined frequency is shown at the right. *KIR A* haplotypes are shaded in red, *KIR B* haplotypes are shaded in blue. **(C)** Shown are the *telomeric KIR* haplotypes identified in five Iranian populations. The combined frequency is shown at the right. *KIR A* haplotypes are shaded in red, *KIR B* haplotypes are shaded in blue. Yellow shading indicates novel *KIR* alleles identified in this study, which are described in **Figure 2**. **(D)** Shows the *HLA class I* allele frequencies observed in the Baloch population.

REFERENCES

1. Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat Rev Immunol.* (2013) 13:133–44. doi: 10.1038/nri3370
2. Vance RE, Eichberg MJ, Portnoy DA, Raule, D. Listening to each other: infectious disease and cancer immunology. *Sci Immunol.* (2017) 2:eaai9339. doi: 10.1126/sciimmunol.aai9339
3. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu Rev Immunol.* (2013) 31:227–58. doi: 10.1146/annurev-immunol-020711-075005
4. Lam VC, Lanier LL. NK cells in host responses to viral infections. *Curr Opin Immunol.* (2017) 44:43–51. doi: 10.1016/j.coi.2016.11.003
5. Stewart CA, Vivier E, Colonna M. Strategies of natural killer cell recognition and signaling. *Curr Top Microbiol Immunol.* (2006) 298:1–21. doi: 10.1007/3-540-27743-9_1
6. Moretta L, Pietra G, Montaldo E, Vacca P, Pende D, Falco M, et al. Human NK cells: from surface receptors to the therapy of leukemias and solid tumors. *Front Immunol.* (2014) 5:87. doi: 10.3389/fimmu.2014.00087
7. Bashirova AA, Martin MP, McVicar, DW, Carrington M. The killer immunoglobulin-like receptor gene cluster: tuning the genome for defense. *Annu Rev Genomics Hum Genet.* (2006) 7:277–300. doi: 10.1146/annurev.genom.7.080505.115726
8. Locatelli F, Pende D, Falco M, Della Chiesa M, Moretta A, Moretta L. NK cells mediate a crucial graft-versus-leukemia effect in haploidentical-HSCT to cure high-risk acute leukemia. *Trends Immunol.* (2018) 39:577–90. doi: 10.1016/j.it.2018.04.009
9. Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci USA.* (2000) 97:4778–83. doi: 10.1073/pnas.080588597
10. Uhrberg M, Valiante NM, Shum BP, Shilling H, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. *Immunity.* (1997) 7:753–63. doi: 10.1016/S1074-7613(00)80394-5
11. Hsu KC, Liu XR, Selvakumar A, Mickelson E, O'Reilly R, Dupont B. Killer Ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. *J Immunol.* (2002) 169:5118–29. doi: 10.4049/jimmunol.169.9.5118
12. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D. Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. *Tissue Antigens.* (2003) 62:79–86. doi: 10.1034/j.1399-0039.2003.00072.x
13. Kikuchi-Maki A, Catina TL, Campbell KS. Cutting edge: KIR2DL4 transduces signals into human NK cells through association with the Fc receptor gamma protein. *J Immunol.* (2005) 174:3859–63. doi: 10.4049/jimmunol.174.7.3859
14. Miah SM, Hughes TL, Campbell KS. KIR2DL4 differentially signals downstream functions in human NK cells through distinct structural modules. *J Immunol.* (2008) 180:2922–32. doi: 10.4049/jimmunol.180.5.2922
15. Rajagopalan S, Long EO. KIR2DL4 (CD158d): an activation receptor for HLA-G. *Front Immunol.* (2012) 3:258. doi: 10.3389/fimmu.2012.00258
16. Guethlein LA, Norman PJ, Hilton HH, Parham P. Co-evolution of MHC class I and variable NK cell receptors in placental mammals. *Immunol Rev.* (2015) 267:259–82. doi: 10.1111/imr.12326
17. Pyo CW, Guethlein LA, Vu Q, Wang R, Abi-Rached L, Norman PJ, et al. Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. *PLoS ONE.* (2010) 5:e15115. doi: 10.1371/journal.pone.0015115
18. Cooley S, Weisdorf DJ, Guethlein LA, Klein J, Wang T, Le CT, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood.* (2010) 116:2411–9. doi: 10.1182/blood-2010-05-283051
19. Oevermann L, Michaelis SU, Mezger M, Lang P, Toporski J, Bertaina A, et al. KIR B haplotype donors confer a reduced risk for relapse after haploidentical transplantation in children with all. *Blood.* (2014) 124:2744–7. doi: 10.1182/blood-2014-03-565069
20. Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J Immunol.* (1998) 161:571–7.
21. Pando MJ, Gardiner CM, Gleimer M, McQueen KL, Parham P. 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 0 182 in Ig domain 1. *J Immunol.* (2003) 171:6640–9. doi: 10.4049/jimmunol.171.12.6640
22. Moesta AK, Norman PJ, Yawata M, Yawata N, Gleimer M, Parham P. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. *J Immunol.* (2008) 180:3969–79. doi: 10.4049/jimmunol.180.6.3969
23. Bari R, Bell T, Leung WH, Vong QP, Chan WK, Das Gupta N. Significant functional heterogeneity among KIR2DL1 alleles and a pivotal role of arginine 245. *Blood.* (2009) 114:5182–90. doi: 10.1182/blood-2009-07-231977
24. VandenBussche CJ, Mulrooney TJ, Frazier WR, Dakshanamurthy S, Hurley CK. Dramatically reduced surface expression of NK cell receptor KIR2DS3 is attributed to multiple residues throughout the molecule. *Genes Immun.* (2009) 10:162–73. doi: 10.1038/gene.2008.91
25. Hilton HG, Guethlein LA, Goyos A, Nemat-Gorgani N, Bushnell D, Norman PJ, et al. Polymorphic HLA-C receptors balance the functional characteristics of KIR haplotypes. *J Immunol.* (2015) 195:3160–70. doi: 10.4049/jimmunol.1501358
26. Gendzekhadze K, Norman PJ, Abi-Rached L, Graef T, Moesta A, Layrisse Z, et al. 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.* (2009) 106:18692–7. doi: 10.1073/pnas.0906051106
27. Norman PJ, Hollenbach JA, Nemat-Gorgani N, Guethlein LA, Hilton HG, Pando MJ, et al. Co-evolution of human leukocyte antigen (HLA) class I ligands with killer-cell immunoglobulin-like receptors (KIR) in a genetically diverse population of sub-Saharan Africans. *PLoS Genet.* (2013) 9:e1003938. doi: 10.1371/journal.pgen.1003938

28. Nemat-Gorgani N, Hilton HG, Henn BM, Lin M, Gignoux CR, Myrick JW, et al. Different selected mechanisms attenuated the inhibitory interaction of KIR2DL1 with C2(+) HLA-C in two indigenous human populations in Southern Africa. *J Immunol.* (2018) 200:2640–55. doi: 10.4049/jimmunol.1701780
29. Nemat-Gorgani N, Guethlein LA, Henn BM, Norberg S, Chiaroni J, Sikora M, et al. Diversity of KIR, HLA class I, their interactions in seven populations of sub-Saharan Africans. *J Immunol.* (2019) 202:2636–47. doi: 10.4049/jimmunol.1801586
30. Middleton D, Meenagh A, Gourraud PA. KIR haplotype content at the allele level in 77 Northern Irish families. *Immunogenetics.* (2007) 59:145–58. doi: 10.1007/s00251-006-0181-7
31. Vierra-Green C, Roe D, Hou L, Hurley CK, Rajalingam R, Reed E, et al. Allele-level haplotype frequencies and pairwise linkage disequilibrium for 14 KIR loci in 506 European-American individuals. *PLoS ONE.* (2012) 7:e47491. doi: 10.1371/journal.pone.0047491
32. Yawata M, Yawata N, Draghi M, Little AM, Partheniou F, Parham P. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. *J Exp Med.* (2006) 203:633–45. doi: 10.1084/jem.20051884
33. Nemat-Gorgani N, Edinur HA, Hollenbach JA, Traherne J, Dunn PP, Chambers GK, et al. KIR diversity in Maori and Polynesians: populations in which HLA-B is not a significant KIR ligand. *Immunogenetics.* (2014) 66:597–611. doi: 10.1007/s00251-014-0794-1
34. Ashouri E, Norman PJ, Guethlein LA, Han A, Nemat-Gorgani N, Norberg SJ, et al. HLA class I variation in Iranian Lur and Kurd populations: high haplotype and allotype diversity with an abundance of KIR ligands. *HLA.* (2016) 88:87–99. doi: 10.1111/tan.12852
35. Cella M, Longo A, Ferrara GB, Strominger JL, Colonna M. NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. *J Exp Med.* (1994) 180:1235–42. doi: 10.1084/jem.180.4.1235
36. Saunders PM, Pymm P, Pietra G, Hughes VA, Hitchen C, O'Connor GM, et al. Killer cell immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of HLA class I recognition. *J Exp Med.* (2016) 213:791–807. doi: 10.1084/jem.20152023
37. Norman PJ, Hollenbach JA, Nemat-Gorgani N, Marin WM, Norberg SJ, Ashouri E, et al. Defining KIR and HLA class I genotypes at highest resolution via high-throughput sequencing. *Am J Hum Genet.* (2016) 99:375–91. doi: 10.1016/j.ajhg.2016.06.023
38. Langmead B, Salzberg S L. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* (2012) 9:357–9. doi: 10.1038/nmeth.1923
39. Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Muller W, Wetter T, et al. Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Res.* (2004) 14:1147–59. doi: 10.1101/gr.1917404
40. Bonfield JK, Whitwham A. Gap5–editing the billion fragment sequence assembly. *Bioinformatics.* (2010) 26:1699–703. doi: 10.1093/bioinformatics/btq268
41. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinformatics.* (2013) 14:178–92. doi: 10.1093/bib/bbs017
42. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet.* (2003) 73:1162–9. doi: 10.1086/379378
43. Cavalli-Sforza LL, Edwards AW. Phylogenetic analysis. models and estimation procedures. *Am J Hum Genet.* (1967) 19(3 Pt 1):233–57. doi: 10.2307/2406616
44. Misra MK, Augusto DG, Martin GM, Nemat-Gorgani N, Sauter J, Hofmann JA, et al. Report from the killer-cell immunoglobulin-like receptors (KIR) component of the 17th international hla and immunogenetics workshop. *Hum Immunol.* (2018) 79:825–33. doi: 10.1016/j.humimm.2018.10.003
45. Norman PJ, Abi-Rached L, Gendzekhadze K, Hammond JA, Moesta A, Sharma D, et al. Meiotic recombination generates rich diversity in NK cell receptor genes, alleles, and haplotypes. *Genome Res.* (2009) 19:757–69. doi: 10.1101/gr.085738.108
46. Hollenbach JA, Nocedal I, Ladner MB, Single RM, Trachtenberg EA. Killer cell immunoglobulin-like receptor (KIR) gene content variation in the HGDP-CEPH populations. *Immunogenetics.* (2012) 64:719–37. doi: 10.1007/s00251-012-0629-x
47. Wagner I, Schefzyk D, Pruschke J, Schofl G, Schone B, Gruber N, et al. Allele-level KIR genotyping of more than a million samples: workflow, algorithm, and observations. *Front Immunol.* (2018) 9:2843. doi: 10.3389/fimmu.2018.02843
48. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG, et al. The IPD and IMGT/HLA database: allele variant databases. *Nucleic Acids Res.* (2015) 43:D423–31. doi: 10.1093/nar/gku1161
49. Martin MP, Bashirova A, Traherne J, Trowsdale J, Carrington M. Cutting edge: expansion of the KIR locus by unequal crossing over. *J Immunol.* (2003) 171:2192–5. doi: 10.4049/jimmunol.171.5.2192
50. Traherne JA, Martin M, Ward R, Ohashi M, Pellett F, Gladman D, et al. Mechanisms of copy number variation and hybrid gene formation in the KIR immune gene complex. *Hum Mol Genet.* (2010) 19:737–51. doi: 10.1093/hmg/ddp538
51. Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, et al. Human NK cell education by inhibitory receptors for MHC class I. *Immunity.* (2006) 25:331–42. doi: 10.1016/j.immuni.2006.06.013
52. Goodridge JP, Jacobs B, Saetersmoen ML, Clement D, Hammer Q, Clancy T, et al. Remodeling of secretory lysosomes during education tunes functional potential in NK cells. *Nat Commun.* (2019) 10:514. doi: 10.1038/s41467-019-08384-x
53. Norman PJ, Abi-Rached L, Gendzekhadze K, Korbel D, Gleimer M, Rowley D, et al. Unusual selection on the KIR3DL1/S1 natural killer cell receptor in Africans. *Nat Genet.* (2007) 39:1092–9. doi: 10.1038/ng2111
54. Taner SB, Pando MJ, Roberts A, Schellekens J, Marsh S, Malmberg KJ, et al. Interactions of NK cell receptor KIR3DL1*004 with chaperones and conformation-specific antibody reveal a functional folded state as well as predominant intracellular retention. *J Immunol.* (2011) 186:62–72. doi: 10.4049/jimmunol.0903657
55. Broushaki F, Thomas MG, Link V, Lopez S, van Dorp L, Kirsanow K, et al. Early neolithic genomes from the eastern Fertile Crescent. *Science.* (2016) 353:499–503. doi: 10.1126/science.aaf7943
56. Gumperz JE, Litwin V, Phillips JH, Lanier LL, Parham P. 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.* (1995) 181:1133–44. doi: 10.1084/jem.181.3.1133
57. Carr WH, Pando MJ, Parham P. KIR3DL1 polymorphisms that affect NK cell inhibition by HLA-Bw4 ligand. *J Immunol.* (2005) 175:5222–9. doi: 10.4049/jimmunol.175.8.5222
58. Ohno S, Aoki K, Sugiura S, Nakayama E, Itakura K, Aizawa M. Letter: HL-A5 and Behcet's disease. *Lancet.* (1973) 2:1383–4. doi: 10.1016/S0140-6736(73)93343-6
59. Verity DH, Wallace GR, Vaughan RW, Stanford M. Behcet's disease: from Hippocrates to the third millennium. *Br J Ophthalmol.* (2003) 87:1175–83. doi: 10.1136/bjo.87.9.1175
60. Petrushkin H, Norman PJ, Lougee E, Parham P, Wallace G, Stanford MR, et al. KIR3DL1/S1 allotypes contribute differentially to the development of behcet disease. *J Immunol.* (2019) 203:1629–35. doi: 10.4049/jimmunol.1801178
61. Sheikh NS, Sheikh AS, Sheikh AA, Yahya S, Rafi-U-Shan, Lateef M. Seroprevalence of hepatitis B virus infection in Balochistan Province of Pakistan. *Saudi J Gastroenterol.* (2011) 17:180–4. doi: 10.4103/1319-3767.80380
62. Rahimi Foroushani A, Farzianpour F, Tavana A, Rasouli J, Hosseini S. The 10-year trend of TB rate in West Azerbaijan Province, Iran from 2001 to 2010. *Iran J Public Health.* (2014) 43:778–86.
63. WHO. *Global Tuberculosis Report 2019*. Geneva: World Health Organization (2019).
64. Mbiribindi B, Mukherjee S, Wellington D, Das J, Khakoo SI. Spatial clustering of receptors and signaling molecules regulates NK cell response to peptide repertoire changes. *Front Immunol.* (2019) 10:605. doi: 10.3389/fimmu.2019.02370
65. Sim MJW, Rajagopalan S, Altmann DM, Boyton RJ, Sun PD, Long EO. Human NK cell receptor KIR2DS4 detects a conserved bacterial epitope presented by HLA-C. *Proc Natl Acad Sci USA.* (2019) 116:201903781. doi: 10.1073/pnas.1903781116
66. Abi-Rached L, Jobin MJ, Kulkarni S, McWhinnie A, Dalva K, Gragert L, et al. The shaping of modern human immune systems by multiregional admixture with archaic humans. *Science.* (2011) 334:89–94. doi: 10.1126/science.1209202

67. Mehrjoo Z, Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Ardalani F, et al. Distinct genetic variation and heterogeneity of the Iranian population. *PLoS Genet*. (2019) 15:e1008385. doi: 10.1371/journal.pgen.1008385
68. Ashouri E, Farjadian S, Reed EF, Ghaderi A, Rajalingam R. KIR gene content diversity in four Iranian populations. *Immunogenetics*. (2009) 61:483–92. doi: 10.1007/s00251-009-0378-7
69. Tajik N, Shahsavar F, Mousavi T, Radjabzadeh MF. Distribution of KIR genes in the Iranian population. *Tissue Antigens*. (2009) 74:22–31. doi: 10.1111/j.1399-0039.2009.01263.x
70. Hiby SE, Ashrafian-Bonab M, Farrell L, Single RM, Balloux F, Carrington M, et al. Distribution of killer cell immunoglobulin-like receptors (KIR) and their HLA-C ligands in two Iranian populations. *Immunogenetics*. (2010) 62:65–73. doi: 10.1007/s00251-009-0408-5
71. Solgi G, Ghafari H, Ashouri E, Alimoghdam K, Rajalingam R, Amirzargar A. (2011). Comparison of KIR gene content profiles revealed a difference between northern and southern Persians in the distribution of KIR2DS5 and its linked loci. *Hum Immunol*. 72:1079–83. doi: 10.1016/j.humimm.2011.08.002

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