

Distribution of killer cell immunoglobulin-like receptors (*KIR*) and their HLA-C ligands in two Iranian populations

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Abstract Killer cell immunoglobulin-like receptors (*KIR*) gene frequencies vary between populations and contribute to functional variation in immune responses to viruses, autoimmunity and reproductive success. This study describes the frequency distribution of 12 variable *KIR* genes and their HLA-C ligands in two Iranian populations who have lived for many generations in different environments: the Azerbaijanis at high altitude and the Jonobi people at sea level. The results are compared with those published for other human populations and a large group of

English Caucasians. Differences were seen in *KIR* and *HLA-C* group frequencies, in linkage disequilibrium and inhibitory/activating *KIR* ratios between the groups. Similarities with geographically close populations in the frequencies of the *KIR* A and B haplotypes and *KIR* AA genotype reflected their common ancestry. The extreme variability of the *KIR* gene family and their HLA-C ligands is highlighted and their importance in defining differences between geographically and culturally isolated communities subject to different environmental pressures who come from the same ethnic grouping.

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Introduction

Natural killer (NK) cells are essential components of the innate immune system and act in the first line of defence against infection, play a role in tumour surveillance and are involved in placental development. Functionally, they are controlled by activating and inhibitory receptors (Colucci et al. 2003; Lanier 2008; McQueen and Parham 2002) and in humans the Killer cell Immunoglobulin-like Receptors (*KIR*) are of central importance in distinguishing normal healthy self cells from diseased or allogeneic targets. HLA class I molecules are ligands for some of the *KIR* and can trigger signals to inhibit effector function of the NK cells or conversely to stimulate killing or cytokine production (Colonna et al. 2000; Long et al. 2001). The *KIR* genes and the genes for their HLA class I ligands reside on different chromosomes and probably constitute the most diverse loci in the human genome. Indeed, the *KIR* region is clearly undergoing rapid evolution and selection and only

the most closely related individuals will share identical *KIR* genotypes (Khakoo et al. 2000; Parham 2005; Shilling et al. 2002). The diversity of the *KIR* region is achieved by both polymorphism at individual *KIR* gene loci as well as variable gene content. The framework *KIR* genes (*3DL3*, *2DL4* and *3DL2*) are present in nearly all individuals. Thirteen additional *KIR* genes have been characterised encoding five inhibitory receptors (*3DL1*, *2DL1–3* and *2DL5*) and six activating *KIR* (*2DS1–5*, *3DS1*). There are also two pseudogenes (*2DP1* and *3DP1*) that encode no cell surface receptors. Although *KIR* genotypes can vary markedly, these can be simplified into two broad categories comprised of A and/or B haplotypes. The simpler A haplotype has 7 *KIR* genes mainly coding for the inhibitory type (*3DL1–3*, *2DL1* and *2DL3*) and a single *KIR* gene, *2DS4*, coding for an activating receptor. *KIR2DS4* is frequently represented by alleles with a frame-shifting deletion that abrogates the activating function of the receptor. The B haplotype has a more variable content with additional *KIR* genes (*2DS1–3*, *2DS5*, *3DS1*, *2DL2* and *2DL5*) chiefly coding for activating receptors. In addition, the B haplotype can be divided into two halves, which are centromeric or telomeric to the centrally placed framework locus, *2DL4* (Yawata et al. 2002). These two halves were deduced from linkage disequilibrium studies and appear to be under differing selection pressures (Yawata et al. 2006). Similarly the numerous HLA-C alleles fall into one of two categories, C1 and C2, as recognized by the NK cell through the *KIR* receptors.

A number of studies of human populations world-wide have shown differences in *KIR* frequencies and haplotypes correlating with ethnicity, migration routes out of Africa and relative population isolation (Denis et al. 2005; Gendzekhadze et al. 2006; Kulkarni et al. 2008; Norman et al. 2002; Rajalingam et al. 2008; Rayes et al. 2008; Single et al. 2007). In the Middle East, the *KIR* frequencies are similar to those found in Caucasian populations whilst *KIR* genotypes in Indians or Pakistanis show a tendency towards an increase in *KIR* B haplotypes. Interestingly, the Parsis who migrated from Persia to North India over 1,000 years ago differ from their North Indian neighbours but show close similarities to *KIR* genotypes in Maharashtrians who settled in North India in ancient times and have their own language (Kulkarni et al. 2008). Until recently there had been no data available for Iranian populations which left a gap, geographically speaking, between the near-East (Mahfouz et al. 2006; Rayes et al. 2008) and Pakistan and India (Kulkarni et al. 2008; Norman et al. 2002; Rajalingam et al. 2002) in reported *KIR* variation.

We have now genotyped samples from two distinct Iranian populations the Azerbaijanis and the Jonobi people and compared our results with those populations that are

geographical neighbours as well as an English Caucasian group. The Azerbaijanis have lived for many generations at high altitude (above 2,400 m) in East Azerbaijan and Ardabil provinces. The Jonobi people from Hormozgan province and Balochistan live at sea level along the shores of the Persian Gulf and Oman Sea (Fig. 1). In epidemiological studies, *KIR* and HLA ligand combinations have been shown to be important in the pathogenesis of certain viral infections such as HIV and HCV (Khakoo et al. 2004; Martin et al. 2002), in autoimmune diseases (Gardiner 2008; Nelson et al. 2004) and in reproductive fitness (Hiby et al. 2004, 2008; Parham 2004). We therefore typed our two Iranian populations for the 12 *KIR* genes characterised by their variable presence and also identified the HLA-C groups, *KIR* ligands C1 and C2, carried by each individual. The Iranian results were compared to our Caucasian population and to other geographically close populations described in the literature.

Materials and methods

Study subjects

Blood samples were collected from unrelated adult males from two isolated Iranian communities as described (Ashrafiyan-Bonab et al. 2007). Marrying out of these communities is not common practice and they are very



Fig. 1 Ethnographic regions of Iran showing the two populations genotyped for *KIR* and HLA-C groups. Borders in the figure roughly correspond to cultural regions based on ethnicity and language. From Ashrafiyan-Bonab et al. 2007. Azerbaijanis traditionally live at >2,400 m, the Jonobi live at sea level in the Hormozgan and Baluchistan provinces

different in terms of culture and language. Samples were randomly collected from consenting volunteers to ensure an unbiased sampling strategy. Ethnic information was obtained on the place of birth of the subject and the place of birth of their parents and grandparents (Ashrafiyan-Bonab et al. 2007). One hundred samples from both populations were analysed in this study. Ethical approval was obtained from the Iranian Ministry of Health and the Iranian Cultural Heritage Organization.

Blood samples were taken from 584 healthy English Caucasian primiparous women and their male partners. Informed written consent and Local Ethics Committees' approval were obtained.

DNA isolation and genotyping

DNA from the Iranian samples was extracted using the PAXgene blood DNA kit (Preanalytix, GmbH, Germany). Genomic DNA from the Caucasian cohort was isolated from 5 ml of blood using the QIAamp DNA Maxi Blood Kit (Qiagen, Crawley, UK). PCR-SSP was used to amplify the genomic DNA for presence or absence of the 12 variable *KIR* genes coding for the inhibitory *KIR*, 2DL1, 2DL2, 2DL3, 2DL5 and 3DL1 and the activating *KIR*, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1 and the pseudogene, 2DP1. *KIR2DS4* alleles were also identified as being of the full-length type or having the 22bp deletion that would negate cell-surface expression, sometimes referred to as *KIR1D*. Two pairs of PCR primers were selected for each gene or allele to give relatively short amplicons of 100 to 800bp as previously described (Hiby et al. 2004). *HLA-C* was also genotyped as group C1 and/or group C2 as recognised by NK cells through the *KIR* receptors. The method used to define these groups by PCR-SSP was as previously described (Hiby et al. 2008).

Data analysis and statistical methods

Carrier frequency was calculated by direct counting of individuals possessing at least one copy of the gene (individuals heterozygous for a *KIR* gene cannot be distinguished from those that are homozygous without family members, who were not available for this study). *KIR* gene frequency estimates were calculated using the equation $KGF = 1 - \sqrt{1 - f}$ where f is the carrier frequency. Frequency of A and B haplotypes was deduced by defining the A haplotype as having the variable genes 2DL1-2DL3-3DL1-2DS4 only. The presence of the B haplotype would then be recognised by detection of any of the other *KIR* genes. Fisher's exact test was used to calculate p values for the comparison of carrier frequencies. LD coefficients for two-locus associations were calculated for unrelated Azerbaijani and Jonobi individuals according

to Imanishi et al. (1991). The statistical significance of LD was calculated using Fisher's exact test. *KIR2DL2/2DL3* and *KIR3DL1/3DS1* allelic pairs and the *HLA-C* groups, C1 and C2, were found to be in Hardy–Weinberg equilibrium in all three populations.

Results and discussion

KIR and *HLA-C* group carrier frequencies

Only those *KIR* genes ($n=12$) that were known to have variable occurrence were typed and thus a measure of gene content could be obtained for each individual. The framework genes, *KIR3DL3*, *3DL2*, and *2DL4* as well as the pseudogene *3DP1* were assumed to be present in all individuals as seen for most other populations. Carrier and estimated gene frequencies are given in Table 1 together with *KIR* frequencies from geographically close populations taken from the literature and from our English Caucasian cohort. The prevalence of the most common *KIR* AA genotype and that of the *HLA-C2* group is also given.

Notably, there were higher frequencies of a number of *KIR*; both those coding for inhibitory receptors, *KIR2DL1* and *KIR3DL1* and activating receptors, *KIR2DS1*, *2DS4* and *3DS1* in the Azerbaijanis compared with the Jonobi population. The genes coding for activating *KIR*, *2DS1* and *3DS1* are in strong linkage disequilibrium to each other and have been implicated in resistance to infection and in reproductive success (Hiby et al. 2008; Martin et al. 2002; Qi et al. 2006). The *KIR2DS4* allele coding for the full-length cell surface receptor was present at equal frequency in the two populations (carrier frequency being 29% in Azerbaijanis, 28% in Jonobis, not shown), the deleted form was more frequent in the Azerbaijanis. Expression of inhibitory *KIR* has been shown to be important in determining the responsiveness of NK cells to stimuli (Anfossi et al. 2006; Yawata et al. 2008).

Overall the Jonobi population more closely resembled the more westerly Palestinian and Lebanese people than did the Azerbaijanis but neither had such high frequencies of the activating *KIR* genes as seen in the more easterly populations of the Karachi South Asians and the Parsis.

Notably *KIR2DS3* was present at high frequency in both Iranian populations (42% carriers) compared to our Caucasian population (30% carriers). This relatively high frequency of *2DS3* was also observed for neighbouring population groups from Palestine (Norman et al. 2001) to the Parsis in India (Kulkarni et al. 2008).

The *KIR* A and B haplotypes were deduced from the individual *KIR* gene frequencies as described. Overall there was a balance in the frequencies of the A and B haplotypes

Table 1 Comparison of observed KIR frequencies (carriers) and estimated KIR gene frequencies with HLA-C2 group frequency in the Iranian, other Asian populations and an English Caucasian cohort

Population	KIR gene	2DL1	2DL2	2DL3	2DL5	3DL1	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DP1	KIRAA genotype%	HLA-C2 group %
Caucasoid ^a (n=584)	Carrier frequency (%)	96.2	52.9	89.7	55.5	94.4	43.6	53.4	29.5	94.3	36.3	44.4	96.2	27.6	32.6
	Estimated gene frequency	.83	.31	.72	.33	.78	.25	.31	.15	.78	.21	.26	.83		
Palestine ^b (n=105)	Carrier frequency %	97	62	85	63	88	44	64	37	88	27	39	ND	23	24
	Estimated gene frequency	.83	.38	.61	.39	.65	.25	.40	.21	.65	.14	.22			
Lebanese ^b (n=120)	Carrier frequency (%)	99	60	88	ND	96	41	59	38	95	31	36	ND	26	31
	Estimated gene frequency	.91	.36	.66		.80	.23	.36	.21	.78	.17	.20			
Jonobi ^a (n=100)	Carrier frequency (%)	97	54	85	62	93	42	57	42	93	36	37	97	26	57
	Estimated gene frequency	.83	.32	.61	.38	.74	.24	.34	.24	.74	.20	.21	.83		
Azerbaijani ^a (n=100)	Carrier frequency (%)	99	56	84	64	97	49	58	42	97	38	47	99	23	44
	Estimated gene frequency	.91	.34	.60	.39	.83	.29	.35	.24	.83	.21	.27	.91		
Karachi S. Asians ^c (n=78)	Carrier frequency (%)	90	67	91	78	81	60	69	45	72	48	56	ND	11.5	
	Estimated gene frequency	.68	.42	.70	.53	.56	.37	.45	.26	.47	.28	.34			
Parsis ^d (n=145)	Carrier frequency (%)	99.3	62.8	81.9	71.0	87.4	62.8	62.8	53.8	86.1	51.0	62.2	99.3	24.1	47.4
	Estimated gene frequency	.92	.39	.58	.46	.65	.39	.39	.32	.63	.30	.39	.92		
N Indians ^e (n=72)	Carrier frequency (%)	87.5	79.2	65.3	79.2	87.5	54.2	62.5	43.1	80.6	47.2	38.9	ND	5.6	31.3
	Estimated gene frequency	.65	.54	.41	.54	.65	.32	.39	.25	.56	.73	.22			

^a Populations genotyped by the authors^b Norman et al. 2001, 2002, Mahfouz et al. 2006^c Norman et al. 2002^d Kulkarni et al. 2008^e Rajalingam et al. 2002. (Estimated gene frequency from $GF = 1 - \sqrt{1 - f}$ where f is the carrier frequency)

as has been reported in all populations analysed (Rajalingam et al. 2008). The Jonobi people had 56% A haplotypes and the Azerbaijanis, 53%. Our Caucasian population had 59% A haplotypes to 41% B haplotypes. From the ratio of frequencies of inhibitory (*2DL1*, *2DL2*, *2DL3*, *3DL1*) to activating (*2DS1*, *2DS2*, *2DS4* full length, *3DS1*) *KIR* genes reported to have HLA class I ligands the Azerbaijanis are deviated more towards activation (ratio inhibitory to activating *KIR* 1.85/1) than the Jonobi people (ratio 2.01/1). The Caucasians gave a ratio with these *KIR* very similar to the Jonobi population of 2.04/1.

In addition there was a significantly higher frequency of the *KIR* ligand HLA-C2 in the Jonobis as compared to the Azerbaijanis (p value, 0.006) and the Caucasians ($p < 0.001$) with a notably high carrier frequency of 57% (Table 1). The C2 frequency is also high in Parsis (47%). In addition, the data from our three populations fits well with the model of Single et al. (2007) who depicted a negative correlation between *KIR2DS1* and its ligand HLA-C2 that provides good evidence for their co-evolution (Fig. 2).

Since writing this report, two publications on other Iranian populations have been released (Ashouri et al. 2009

and Tajik et al. 2009). One reported on the general Iranian population selected from a number of cities (Tajik et al. 2009) and the second includes an Azeri population that was not selected on living at high altitude and indeed does not resemble our own Azerbaijani population in terms of the *KIR* frequencies (Ashouri et al. 2009). The possible confounding effect of racial admixture was suggested for this latter population by the authors.

KIR genotype profiles

The Azerbaijanis and Jonobi people shared 16 *KIR* genotype profiles and 15 of these were also seen in the Caucasian population (Fig. 3). The *KIR* AA genotype was the most frequent in all three populations. The Azerbaijanis had seven profiles that were not found in the Jonobi people (five of these were seen in the Caucasians). In addition, the Jonobi people possessed 12 profiles not found in the Azerbaijanis and only four of these were seen in the Caucasians. The English Caucasian population had an additional 18 *KIR* profiles that were not seen in either Iranian population and these were uncommon genotypes present in one to four individuals only. The number of distinct profiles in the Azerbaijani, Jonobi and Caucasians numbered 23, 28 and 43, respectively. This would support the view that the Iranian populations were from relatively isolated populations as seen with the Parsis (19 profiles, Kulkarni et al. 2008) and in contrast to the genetically diverse North Indians (47 profiles, Rajalingam et al. 2002) and our Caucasian population (Shifman et al. 2003).

KIR A and B haplotypes

Unusual *KIR* genotypes, with all the *KIR* A haplotype genes but with single additional genes of the B haplotype were observed. In the Azerbaijani population, two individuals possessed *KIR2DS1* with the A haplotype genes but no other B haplotype *KIR*. This has been observed in other populations at low frequency (Kulkarni et al. 2008). Another Azerbaijani individual had an additional *KIR2DS2* with the *KIR* A haplotype genes as reported in several Indian populations (Rajalingam et al. 2008). In addition a single Jonobi individual had the unusual combination of *KIR* AA genotype genes with a *KIR2DL5* gene. This has not been reported before.

The next most common genotype in the Azerbaijanis after *KIR* AA comprised both an A haplotype and a B haplotype which was characterised by presence of the, mainly activating, telomeric group of *KIR* genes: *3DS1-2DL5-2DS5-2DS1* (profile 2 shared, Fig. 3). This was in contrast to the second most common in the Jonobi which had the A haplotype loci together with the centromeric B

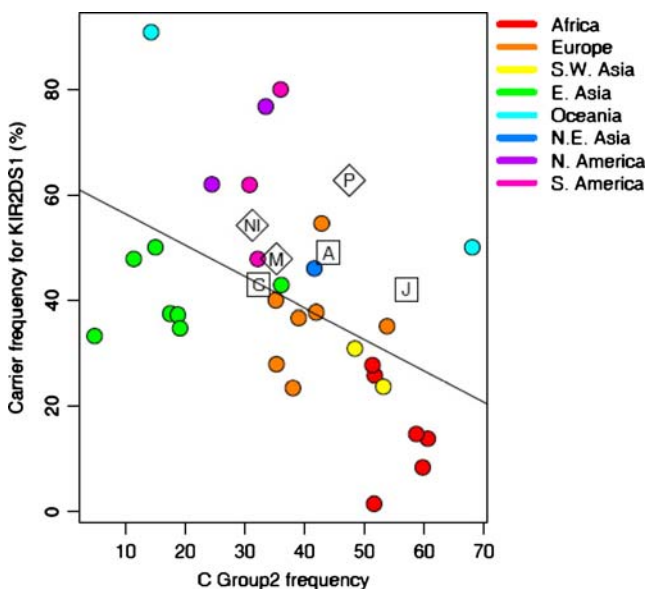


Fig. 2 Correlation between *KIR2DS1* and C-group 2 frequencies. Results for our populations are superimposed upon the global distribution. Each coloured circle represents a distinct population, with the colours identifying the geographic origin of the populations. The solid line represents the estimated regression line (Single et al. 2007). The Iranian and Caucasian population data reported here fits well with the study of Kulkarni et al. (2008) The activating *KIR2DS1* shows a negative correlation with its HLA-C2 ligand although this did not reach significance. Boxed letters denote our populations: C Caucasian, A Azerbaijani and J Jonobi. For comparison, NI North Indian, M Maharashtrian and P Parsis are also shown within the diamonds

Profiles	KIR genes											Azerbaijani	Jonobi	Caucasian
	2DL1	2DL2	2DL3	2DL5	3DL1	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	n=100	n=100	n=584
Profiles shared by Azerbaijanis and Jonobis												%	%	% (n)
1AA												22	26	27.4 (160)
2												14	8	14.0 (82)
3												11	9	13.5 (79)
4												10	12	7.4 (43)
5												6	7	4.5 (26)
6												5	2	1.5 (9)
7												5	4	4.1 (24)
8												4	2	6.7 (39)
9												3	1	1.2 (7)
10												3	3	1.5 (9)
11												2	1	1.7 (10)
12												2	2	1.7 (10)
13												2	3	0.2 (1)
14												1	1	0.2 (1)
15												1	2	0.9 (5)
16												1	1	0.2(1)
Profiles found in Azerbaijanis but not Jonobis														
1												2	0	0
2												1	0	0.7 (4)
3												1	0	0.9 (5)
4												1	0	0
5												1	0	1.2 (7)
6												1	0	0.2 (1)
7												1	0	0.2(1)
Profiles found in Jonobis but not Azerbaijanis														
1												0	3	2.4 (14)
2												0	2	0
3												0	2	1.2 (7)
4												0	1	0
5												0	1	0
6												0	1	0
7												0	1	0
8												0	1	0.3 (2)
9												0	1	0
10												0	1	0
11												0	1	0.7 (4)
12												0	1	0

Fig. 3 KIR genotype profiles and frequencies for the two Iranian and an English Caucasian population. The Caucasian population had 18 unique profiles not seen in the Iranian populations; these were uncommon genotypes present in one to four individuals (not shown here)

haplotype genes *2DL2* and *2DS2* (profile 4 shared, Fig. 3).

Linkage disequilibrium

Comparing linkage disequilibrium between pairs of *KIR* genes in the two Iranian populations reflected their different spread of *KIR* genotypes (Fig. 4). The Jonobi sample had 29 pairs of genes in significant positive or negative LD compared with the Azerbaijani with 22 and this can be compared with the endogamous Parsis population with 39 pairs. The higher frequency of the *KIR* AA genotype in the Jonobi people was reflected in a more marked distinction between the LD values of the haplotype A and B *KIR* genes seen for this population. There were 13 pairs of genes that gave discordant results: eight pairs were in significant negative LD only in the Jonobi (*3DS1-3DL1*, *2DL5-3DL1*, *2DS5-3DL1*, *2DS1-3DL1*, *2DS4-3DS1*, *2DS4-2DL5*, *2DS4-2DS5* and *2DS4-2DS1*), whereas two pairs were in significant positive LD (*2DP1-2DL3* and *2DL1-2DL3*). Only three *KIR* gene pairs in the Azerbaijani showed

significance differences not found in the Jonobi with *2DL3-2DL5*, *2DL3-2DS5* and *2DL3-2DS1* being in significant negative LD. Like the Parsis the Azerbaijani also showed an unusual lack of LD between *KIR2DL3* and *KIR2DL1* which are closely linked on the A haplotype and are reported to be in strong LD in all other published populations. The *KIR2DL1*004* allele that resides on the B haplotype is not in LD with *KIR2DL3* so this may explain the results obtained if the Azerbaijani possess an unusual high frequency of the B haplotype *2DL1* alleles. *KIR2DL1* allele typing would be required to confirm this.

Summary

The two Iranian populations showed distinctive differences in *KIR* and *HLA-C* group carrier and genotype frequencies. Comparison with other genotyped populations to the west and east of Iran can now be made using this data. We also describe unusual *KIR* genotypes emphasising the great diversity and malleability of the *KIR* locus in human

Fig. 4 Linkage disequilibrium analysis for 12 KIR genes in Azerbaijanis and Jonobi people. LD coefficients (*empty triangle*) and *p* values (*p*) on the *upper triangle* are for the Azerbaijanis. Values on the *lower triangle* are for the Jonobis. *NS* not significant

		Azerbaijanis											
		2DS2	2DL2	2DL3	2DP1	2DL1	3DL1	3DS1	2DL5	2DS3	2DS5	2DS1	2DS4
2DS2	Δ		0.218	-0.259	-0.065	-0.065	-0.112	0.018	0.101	0.131	0.009	0.027	-0.112
	p		<0.001	<0.001	NS	NS	NS	NS	<0.001	<0.001	NS	NS	NS
2DL2	Δ	0.211		-0.265	-0.066	-0.066	-0.115	0.027	0.102	0.127	0.016	0.036	-0.115
	p	<0.001		<0.001	NS	NS	NS	NS	<0.001	<0.001	NS	NS	NS
2DL3	Δ	-0.254	-0.263		0.060	0.060	0.031	-0.068	-0.240	-0.163	-0.070	-0.112	0.031
	p	<0.001	<0.001		NS	NS	NS	NS	<0.001	<0.001	0.047	0.006	NS
2DP1	Δ	-0.114	-0.117	0.106		0.090	-0.017	-0.073	-0.060	0.024	-0.079	-0.071	-0.017
	p	NS	NS	0.003		0.01	NS	NS	NS	NS	NS	NS	NS
2DL1	Δ	-0.114	-0.117	0.106	0.143		-0.017	-0.073	-0.060	0.024	-0.079	-0.071	-0.017
	p	NS	NS	0.003	<0.001		NS	NS	NS	NS	NS	NS	NS
3DL1	Δ	0.027	0.044	-0.102	-0.046	-0.046		-0.126	-0.104	-0.132	-0.136	-0.124	0.143
	p	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	<0.001
3DS1	Δ	0.018	0.000	0.009	0.036	0.036	-0.210		0.163	0.054	0.134	0.173	-0.126
	p	NS	NS	NS	NS	NS	<0.001		<0.001	0.015	<0.001	<0.001	NS
2DL5	Δ	0.106	0.092	-0.066	0.066	0.066	-0.163	0.127		0.143	0.128	0.155	-0.104
	p	<0.001	<0.001	NS	NS	NS	0.042	<0.001		<0.001	<0.001	<0.001	NS
2DS3	Δ	0.125	0.124	-0.095	0.041	0.041	-0.028	0.051	0.147		0.000	0.048	-0.132
	p	<0.001	<0.001	0.01049	NS	NS	NS	0.0113	<0.001		NS	0.042	NS
2DS5	Δ	0.023	0.005	-0.027	0.035	0.035	-0.112	0.113	0.123	0.015		0.145	-0.136
	p	NS	NS	NS	NS	NS	0.00831	<0.001	<0.001	NS		<0.001	NS
2DS1	Δ	0.030	0.013	-0.030	0.041	0.041	-0.201	0.130	0.147	0.044	0.132		-0.124
	p	NS	NS	NS	NS	NS	0.002	<0.001	<0.001	0.03983	<0.001		NS
2DS4	Δ	0.027	0.044	-0.102	-0.046	-0.046	0.195	-0.210	-0.163	-0.028	-0.112	-0.201	
	p	NS	NS	NS	NS	NS	<0.001	<0.001	0.042	NS	0.00831	0.002	
		Jonobis											

populations that may arise from selective pressures in response to pathogens, reproductive fitness or other factors in the environment. Our other studies indicate that *KIR B* telomeric genes are those lacking in Caucasian women who experience problems during pregnancy. Of particular interest in this regard are pregnancy and reproduction at high altitude: birth weight decreases with altitude and the risk of foetal growth restriction and pre-eclampsia are increased (Julian et al. 2007; Zamudio 2007). Furthermore, these observations are likely due directly to hypoxia at altitude, rather than economic status (Giussani et al. 2001). Importantly, those with high altitude ancestry are partially protected from this reduc-

tion in fetal growth compared with those who have settled in the area more recently (Julian et al. 2007). When comparing these two Iranian populations for the two polymorphic gene families the increased incidence of *KIR B* telomeric genes in the Azerbaijanis compared to the Jonobis is interesting because the incidence of hypertension in pregnancy and recurrent miscarriage is increased in these Iranians living at high altitude in data supplied by the Iranian Ministry of Health (Table 2). Whether differences in *KIR* can be ascribed to contrasting environments, especially adaptations to living at high and low altitude, can only be determined as data on other similar populations increases.

Table 2 Reproductive problems in the two Iranian populations (data from the Iranian Ministry of Health)

Geographical area Population	East Azerbaijan and Ardabil Azerbaijanis	Hormozghan and Baluchistan Jonobi
Recurrent miscarriage or late pregnancy miscarriage	5.3%	3.2%
Low birth weight<2,500 g	6.9%	6.7%
Hypertension	12.5%	6.8%
Blurred vision and headache due to hypertension	4.9%	2.3%

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References

- Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA et al (2006) Human NK cell education by inhibitory receptors for MHC class I. *Immunity* 25:331–342. doi:10.1016/j.immuni.2006.06.013
- Ashouri E, Farjadian S, Reed EF, Ghaderi A, Rajalingam R (2009) KIR gene content diversity in four Iranian populations. *Immunogenetics* 61:483–492. doi:10.1007/s00251-009-0378-7
- Ashrafian-Bonab M, Lawson Handley LJ, Balloux F (2007) Is urbanization scrambling the genetic structure of human populations? A case study. *Heredity* 98:151–156. doi:10.1038/sj.hdy.6800918
- Colonna M, Moretta A, Vely F, Vivier E (2000) A high resolution view of NK-cell receptors: structure and function. *Immunol Today* 21:428–431. doi:10.1016/S0167-5699(00)01697-2
- Colucci F, Caliguri MA, Di Santo JP (2003) What does it take to make a natural killer. *Nat Rev Immunol* 3:413–425. doi:10.1038/nri1088
- Denis L, Sivula J, Gourraud PA, Kerdoudou N, Chout R, Ricard C, Moisan JP, Gagne K, Partanen J, Bignon JD (2005) Genetic diversity of KIR natural killer cell markers in populations from France, Guadeloupe, Finland, Senegal and Reunion. *Tissue Antigens* 66:267–276. doi:10.1111/j.1399-0039.2005.00473.x
- Gardiner CM (2008) Killer cell immunoglobulin-like receptors on NK cells: the how, where and why. *Int J Immunogenet* 35:1–8. doi:10.1111/j.1744-313X.2007.00739.x
- Gendzekhadze K, Norman PJ, Abi-Rached L, Layrisse Z, Parham P (2006) High KIR diversity in Amerindians is maintained using few gene-content haplotypes. *Immunogenetics* 58:474–480. doi:10.1007/s00251-006-0108-3
- Giussani DA, Phillips PS, Anstee S, Barker DJ (2001) Effects of altitude versus economic status on birth weight and body shape at birth. *Pediatr Res* 49:490–494. PMID:11264431
- Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, Moffett A (2004) Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med* 200:957–965. doi:10.1084/jem.20041214
- Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A (2008) Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum Reprod* 23:972–976. doi:10.1093/humrep/den011
- Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T (1991) Estimation of allele and haplotype frequencies for HLA and complement loci. In: Tsuji K, Aizawa M, Sasazuki T (eds) HLA: 1991 Proceedings of the eleventh International Histocompatibility Workshop and Conference, vol 1. Oxford University Press, New York, 6–13 November 1991
- Julian CG, Vargas E, Armaza JF, Wilson MJ, Niermeyer S, Moore LG (2007) High-altitude ancestry protects against hypoxia-associated reductions in fetal growth. *Arch Dis Child Fetal Neonatal Ed* 92:372–377. doi:10.1136/adc.2006.109579
- Khakoo SI, Rajalingam R, Shum BP, Weidenbach K, Flodin L, Muir DG, Canavez F, Cooper SL, Valiante NM, Lanier LL, Parham P (2000) Rapid evolution of NK receptor systems demonstrated by comparison of chimpanzees and humans. *Immunity* 12:687–698
- Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J et al (2004) HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 305:872–874. doi:10.1126/science.1097670
- Kulkarni S, Single MMP, Rajalingam R, Badwe R, Joshi N, Carrington M (2008) Comparison of the rapidly evolving KIR locus in Parsis and natives of India. *Immunogenetics* 60:121–129. doi:10.1007/s00251-008-0279-1
- Lanier L (2008) Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol* 9:495–502. doi:10.1038/ni1581
- Long EO, Barber DF, Burshtyn DN, Faure M, Peterson M, Rajagopalan S, Renard V, Sandusky M, Stebbins CC, Wagtmann N, Watzl C (2001) Inhibition of natural killer cell activation signals by killer cell immunoglobulin-like receptors (CD158). *Immunol Rev* 181:223–233. doi:10.1034/j.1600-065X.2001.1810119.x
- Mahfouz R, Rayes R, Mahfoud Z, Bazarbachi A, Zaatari G (2006) Distribution of killer cell immunoglobulin-like receptors genotypes in the Lebanese population. *Tissue Antigens* 68:66–71. doi:10.1111/j.1399-0039.2006.00605.x
- Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ et al (2002) Epistatic interaction between KIR3DS1 and HLA-B delays progression to Aids. *Nat Genet* 31:429–434. doi:10.1038/ng934
- McQueen KL, Parham P (2002) Variable receptors controlling activation and inhibition of NK cells. *Curr Opin Immunol* 14:615–621
- Nelson GW, Martin MP, Gladman D, Wade J, Trowsdale J, Carrington M (2004) Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. *J Immunol* 173:4273–4276
- Norman PJ, Stephens HAF, Verity DH, Chandanayingyong D, Vaughan RW (2001) Distribution of natural killer cell immunoglobulin-like receptor sequences in three ethnic groups. *Immunogenetics* 52:195–205. doi:10.1038/sj.gene.6363836
- Norman PJ, Carrington CVF, Byng M, Maxwell LD, Curran MD, Stephens HAF et al (2002) Natural killer cell immunoglobulin-like receptor (KIR) locus profiles in African and South Asian populations. *Genes Immun* 3:86–95. doi:10.1038/sj.gene.6363836
- Parham P (2004) NK cells and trophoblasts: partners in pregnancy. *J Exp Med* 200:951–955. doi:10.1084/jem.20041783
- Parham P (2005) MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol* 5:201–214. doi:10.1038/nri1570
- Qi Y, Mp M, Gao X, Jacobson L, Goedert JJ, Buchbinder S, Kirk GD, O'Brien SJ, Trowsdale J, Carrington M (2006) KIR/HLA pleiotropism: protection against both HIV and opportunistic infections. *PLoS Pathog* 2:e79. doi:10.1371/journal.ppat.0020079
- Rajalingam R, Krausa P, Shilling HG, Stein JB, Balamurugan A, McGinnis MD, Cheng NW, Mehra NK, Parham P (2002) Distinctive KIR and HLA diversity in a panel of north Indian

- Hindus. *Immunogenetics* 53:10009–1019. doi:10.1007/s00251-001-0425-5
- Rajalingam R, Du Z, Meenagh A, Luo L, Kavitha VJ, Pavithra-Arulvani R et al (2008) Distinct diversity of KIR genes in three southern Indian populations: comparison with world populations revealed a link between KIR gene content and pre-historic human migrations. *Immunogenetics* 60:207–217. doi:10.1007/s00251-008-0286-2
- Rayes R, Bazarbachi A, Khazen G, Sabbagh A, Zaatari G, Mahfouz R (2008) Natural killer cell immunoglobulin-like receptors (KIR) genotypes in two Arab populations: will KIR become a genetic landmark between nations? *Mol Biol Rep* 35:225–229. doi:10.1007/s11033-007-9074-6
- Single RM, Martin MP, Gao X, Meyer D, Yeager M, Kidd JR, Kidd KK, Carrington M (2007) Global diversity and evidence for coevolution of KIR and HLA. *Nat Genet* 39:1114–1119. doi:10.1038/ng2077
- Shifman S, Kuypers J, Kokoris M, Yakir B, Darvasi A (2003) Linkage disequilibrium patterns of the human genome across populations. *Hum Mol Genet* 12:771–776
- Shilling HG, Guethlein LA, Cheng NW, Gardiner CM, Rodriguez R, Tyan D, Parham P (2002) Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype. *J Immunol* 168:2307–2315
- Tajik N, Shahsavari F, Mousavi T, Radjabzadeh MF (2009) Distribution of KIR genes in the Iranian population. *Tissue Antigens* 74:22–31. doi:10.1111/j.1399-0039.2009.01263.x
- Yawata M, Yawata N, Abi-Rached L, Parham P (2002) Variation within the human killer cell immunoglobulin-like receptor (KIR) gene family. *Crit Rev Immunol* 22:463–482
- Yawata M, Yawata N, Draghi M, Little AM, Partheniou F, Parham P (2006) Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. *J Exp Med* 203:633–645. doi:10.1084/jem.20051884
- Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P (2008) MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. *Blood* 112:2369–2380. doi:10.1182/blood-2008-03-143727
- Zamudio S (2007) High-altitude hypoxia and preeclampsia. *Front Biosci* 12:2967–2977