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Authors

Osman, Awad E
Mubasher, Mohamed
ElSheikh, Nezar E
et al.

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Brief Communication

Characterization of human killer immunoglobulin-like receptors (KIRs) among healthy Saudis



Awad E. Osman^{a,*}, Mohamed Mubasher^a, Nezar E. ElSheikh^a, Hanan AlHarthi^a, Ahmed S. Al Yami^a, Raja Rajalingam^b, Abdulwahid Al-Dehaimi^a, Derek Middleton^c, Gehad ElGhazali^d

^a King Fahad Medical City, Riyadh 11525, Saudi Arabia

^b UCLA Immunogenetics Centre, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, University of California at Los Angeles, United States

^c Transplant Immunology, Royal Liverpool and Broadgreen University Hospital, Liverpool, UK

^d Mafraq Hospital and Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates

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ABSTRACT

Genes encoding KIRs vary in frequency among different populations and ethnic groups. This study investigated the KIR gene frequency distribution in 148 healthy unrelated Saudi subjects and compared the results with other published findings. All inhibitory and activating KIR genes were present at variable frequencies, with A haplotype-associated genes (KIR2DL1, -2DL3, -3DL1, and KIR2DS4) being observed at higher frequencies (88.9–99.5%) than B haplotype-associated genes (KIR2DS1, -2DS2, -2DS3, -2DS5, -2DL5 and -2DL2) (31.1–70.1%). Thirty-one different KIR genotypes were observed, and AA genotypes displayed the highest frequency (18.2%). This Saudi population possesses similar KIR gene distributional characteristics to those reported in other neighboring populations (e.g., Lebanese) and shows disparities in certain genes and gene contents from other populations (e.g., Australian Aborigines). These findings can be used as a reference control in future studies evaluating the functional significance of the KIR genes and their associations with specific diseases.

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1. Introduction

Human natural killer (NK) cells are a subset of lymphocytes that have the ability to react with cells lacking human leukocyte antigen (HLA) class I without prior immunological exposure [1]. NK cell function is determined by killer immunoglobulin receptors (KIRs) and the type of HLA ligand on the surface of target cells [2]. Binding of KIRs to putative ligands can induce suppression or activation of NK cells. NK cell-mediated cytotoxicity depends on a fine balance between the inhibitory and activating signals induced by KIR molecules on the NK cell surface [3,4].

KIR genes are highly polymorphic and are located on chromosome 19q13.4 [5]. In humans, 16 KIR genes have been identified, defined as the KIR2DL1, -2DL2, -2DL3, -2DL4, -2DL5, -3DL1, -3DL2, -3DL3, -2DS1, -2DS2, -2DS3, -2DS4, -2DS5, -3DS1, -2DP1 and -3DP1 genes. With few exceptions, each KIR gene typically encodes

either an inhibitory or activating molecule [6]. Two groups of KIR haplotypes have been defined, designated A and B based on their KIR gene content [7]. The A haplotype is composed of six inhibitory KIR genes: KIR2DL1, -2DL3, -3DL1, -3DL2, -3DL3 and -2DL4; one activating KIR gene: KIR2DS4; and two pseudogenes KIR2DP1 and -3DP1. The B haplotype differ in its gene content, which is determined mainly by the presence or absence of the genes that are not part of the A haplotype. [5]. The framework KIR3DL2, -3DL3 and -2DL4 genes are present in both the A and B haplotypes, and most activating KIR genes are found within the B haplotype [8].

Full-length sequencing of KIR haplotypes showed that the framework regions divide the KIR locus into two parts: centromeric (C) and telomeric (T) segments that differ in their gene contents [5,9,10]. The centromeric part of the A haplotype contains KIR2DL3, -2DL1 and -2DP1, in addition to 3DL3 and -3DP1, while the telomeric part contains the KIR3DL1 and -2DS4 genes, in addition to KIR2DL4 and -3DL1. Unlike the A haplotype, which is fixed, uniform and conserved, the B haplotype is polymorphic and exhibits a variable centromeric end that includes the KIR2DS2, -2DL2, -2DL5B, -2DS3 and -2DS5 genes, in addition to KIR3DL3

Abbreviations: NK, natural killer; KIR, killer immunoglobulin-like receptor; BMT, bone marrow transplant; SSOP, Sequence-Specific Oligonucleotide Probe.

* Corresponding author.

E-mail address: awadelsid@yahoo.com (A.E. Osman).

and -3DP1, while the telomeric part of the B haplotype contains the KIR3DS1 and -2DS1 genes, in addition to KIR2DL4 and -3DL1 [10,11]. Therefore, diversity is generated in the KIR locus by the content of group A and group B haplotype genes and the allelic variation that occurs within both the centromeric and telomeric regions [12]. KIR allelic products have been shown to differ in their specificity and affinity regarding ligand binding [13], and these allelic differences may contribute to disease susceptibility and progression [14]. The frequencies of KIR genes and haplotypes show great variation among different populations, and the crucial function of KIRs in immunity may have an impact on susceptibility to infection, autoimmune diseases and transplantation outcomes among different ethnic groups [10,15,16].

Saudi Arabia covers 80% of the Arabian Peninsula, mainly in the central arid region. The remaining 20% comprises Yemen, Oman, the United Arab Emirates, Qatar, Bahrain and Kuwait. Some reports have provided support for the “out-of-Africa” hypothesis of human dispersion, which indicates that immigrants followed a southern pathway along the tropical coast of the Arabian Peninsula, India, Southeast Asia and Australia before pursuing the Levantine pathway [11,17]. The geographical location of the Arabian Peninsula played an important role in trade, cultural exchange and warfare following the appearance of the Old World civilizations as well as the emergence of Islam and the subsequent cultural expansion, all of which are factors that may have affected the Arab gene pool. Unlike the population of the coastal area, the central part of the Peninsula has been stable and is less susceptible to migration-related factors [17,18]. The present study aimed to investigate and characterize the KIR genes frequencies and KIR genotype contents of unrelated healthy Saudi subjects and compare the results with other populations.

2. Materials and methods

From the available list of potential bone marrow transplant (BMT) family donors, 148 subjects met the inclusion criteria of being healthy and unrelated. This entailed the exclusion of siblings showing identical HLA matching to the patient. In the event of finding more than one eligible sibling per family, only one sibling was randomly selected for this study. The sample size of 148 afforded the statistical ability to detect at least a 1.5-fold significant difference in the odds ratio vs. other populations in at least one KIR gene. The study received ethical approval from the Institutional Review Board of King Fahad Medical City (KFMC), and informed consent was obtained prior to the time of blood collection. Sequence-Specific Oligonucleotide Probe (SSOP) kits from One Lambda (San Diego, CA, USA) were used for KIR genotyping, and for quality assurance purposes, multiple DNA samples with a known

KIR genotype were run in parallel with our study samples. The same exact results were always obtained for these controls.

2.1. Assigning haplogroup-based genotypes

Individuals carrying one or more of the KIR2DL2, -2DL5, -3DS1, -2DS1, -2DS2, -2DS3 and -2DS5 genes were considered to be of group B haplotypes, and conversely, those who did not carry any of these genes were considered to be of group A haplotypes. Haplotype group ID profiles and the assignment of each individual to an AA or Bx genotype (x can be either an A or B haplotype) were obtained according to <http://www.allelefreqencies.net> [19].

2.2. Centromeric and telomeric genes cluster classification

Based on the presence or absence of the centromeric (C) and telomeric (T) clusters, Bx genotypes are classified into four subsets: C4T4 (presence of both C and T), C4Tx (presence of C and absence of T), CxT4 (absence of C and presence of T) and CxTx (absence of both) [5].

2.3. Statistical analysis

Using the SAS program [20], the frequencies of positive individuals for each of the 16 KIR genes were determined using an algorithm based on a direct counting method. Comparisons with other populations were based on nine KIR genes (KIR2DL1, -2DL3, -2DS4, -3DL1, -2DL2, -2DS1, -2DS2, -2DS3 and -3DS1). For this purpose, Fisher’s exact test and logistic regression methodology were used. To determine significant differences between our study population and other populations, the log of the odds (logit) for the presence of each KIR gene was modeled as a function of each population. The overall significance level was set at 0.05. Principal components analysis (PCA) was conducted using the SAS software [20] to determine and portray the genetic differences and similarities between our study sample and other populations.

3. Results and discussion

3.1. KIR gene frequencies

As explained in Table 1, the framework genes were observed at a frequency of 100% in our sample and all of the compared populations (data obtained from <http://www.allelefreqencies.net>), except for 3DL3 in the Moroccan Chaouya population (97%). Additionally, the inhibitory KIR genes were observed at variable but high frequencies, except for 2DL2 and 2DL5, which were observed at proportionally lower frequencies. Notably, this case for all of the compared populations shown in Table 1, except for the

Table 1
The frequencies of KIR genes in our study population compared to previously published data for Arabs and non-Arab-related populations.

Population	No	2DL1%	2DL3%	2DS4%	3DL1%	2DL2%	2DL5%	2DS1%	2DS2%	2DS3%	2DS5%	3DS1%	2DP1%	2DL4%	3DL2%	3DL3%	3DP1%
Saudis-2 (current)	148	98	83.1	92.5	94.4	68.2	64.9	43.9	73	43.2	43.9	35.8	98	100	100	100	100
Saudis-1	162	96.3	91.4	93.8	95.7	56.2	56.2	33.3	57.4	38.3	27.8	34.6	96	100	100	100	100
Jordanians Palestine	105	83	85	88	88	62	63	44	64	37	27	39	N/R	100	100	N/R	N/R
Lebanese	120	99.2	88.3	95	95.8	59.2	58.3	40.8	59.2	37.5	30.8	35.8	N/R	100	100	N/R	N/R
Omani	99	98	87.9	94.9	96	50.5	59.6	32.3	49.5	30.3	39.4	29.3	98	100	100	100	100
Moroccans Chaouya	67	95.5	73.1	100	100	70.1	67.2	25.4	65.7	52.2	32.8	25	100	100	100	97	100
Tunisians	114	99.1	91.2	96.5	100	59.6	60.5	22.8	59.6	38.6	23.7	23.7	N/R	100	100	100	N/R
Iranians- Arab	76	100	89.5	98.7	85.5	63.1	67.1	42.7	56.3	50	35.5	42.1	98.7	100	100	100	100
Indians Paravar	77	97	79	84	84	69	83	65	75	49	66	60	97	100	100	100	100
Indians Kanikar	35	97	77	80	80	74	86	60	74	63	60	69	94	100	100	100	100
Gabonese	54	100	79.6	100	100	64.8	70.4	18.5	57.4	35.2	33.3	7.4	100	100	100	100	100
Senegalese	118	100	90	100	99	55	52	13	42	24	30	4	100	100	100	100	100
Australian Aborigines	67	72	68	52	55	79	N/R	82	84	81	N/R	78	N/R	N/R	N/R	N/R	N/R

Australian Aborigines. Worldwide, the frequencies of KIR2DL2 and KIR2DL5 are reported to be high among the population of Papua New Guinea (95.5% and 86%, respectively), whereas much lower values are observed for KIR2DL2 among Japanese individuals, at 8.5%, and for KIR2DL5 among Indian Asians from the West Midlands, England, at 26% [19]. In accordance with previous reports, KIR3DL1 and KIR3DS1 in this study were ascertained to be either positive, or one positive while the other negative [1]. The selected populations for comparison in this study were either of Arab origin or part of the populations that followed the southern pathway along the tropical coast of the Arabian Peninsula and India, ending in Australia, according to the out-of-Africa dispersion theory.

As shown from the determination of the significant differences for our study population versus others in Table 2, the Australian

Aborigines significantly segregated themselves across seven out of the nine examined KIR genes frequencies (all *p*-values \leq 0.001). Furthermore, the Senegalese and Indians Kanikar (as well as the Indians Paravar) displayed significant differences in five and four KIR genes, respectively. The Arab-related populations (Saudi-1, Lebanese, Jordanian Palestine, Omani, Moroccan Chaouya, Tunisian and Iran Arab) exhibited statistically significant differences in one to three KIR genes. It is not surprising that the gene frequencies observed in our study population are closer to those found in populations with similar cultures and the same mother tongue. The reason for some of the disparities observed in the frequencies of a few KIR genes for these populations from the current study is not yet clear, but this heterogeneity may reflect the ethnic admixture of these populations.

Table 2
Statistical significance (*p*-value) testing of the KIR genes that were commonly reported across all of the populations compared in this study versus our study population (statistically significant values at the <0.5 level are indicated in **bold**).

Population	2DL1	2DL2	2DL3	2DS1	2DS2	2DS3	2DS4	3DL1	3DS1
Saudis-1	0.38	0.03	0.11	0.07	0.05	0.44	0.82	0.85	0.81
Jordanians Palestine	<.001	0.29	0.91	0.91	0.12	0.38	0.19	0.06	0.59
Lebanese	0.43	0.12	0.36	0.69	0.02	0.41	0.41	0.63	0.99
Omanis	0.54	0.01	0.45	0.08	<.001	0.06	0.45	0.62	0.28
Moroccan Chaouya	0.32	0.77	0.06	0.01	0.28	0.18	0.98	0.98	0.13
Tunisians	0.46	0.15	0.11	<.001	0.02	0.51	0.18	0.97	0.04
Iranians- Arab	0.98	0.44	0.31	0.87	0.01	0.29	0.09	0.03	0.35
Indians Paravar	0.41	0.92	0.12	0.002	0.7	0.43	0.03	0.01	0.001
Indians Kanikar	0.76	0.48	0.31	0.07	0.87	0.03	0.03	0.01	<.001
Gabonese	0.97	0.64	0.41	<.001	0.03	0.35	0.98	0.98	0.001
Senegalese	0.96	0.02	0.21	<.001	<.001	<.001	0.97	0.07	<.0001
Australian Aborigines	<.001	0.11	<.001	<.001	0.09	<.001	<.001	<.001	<.001

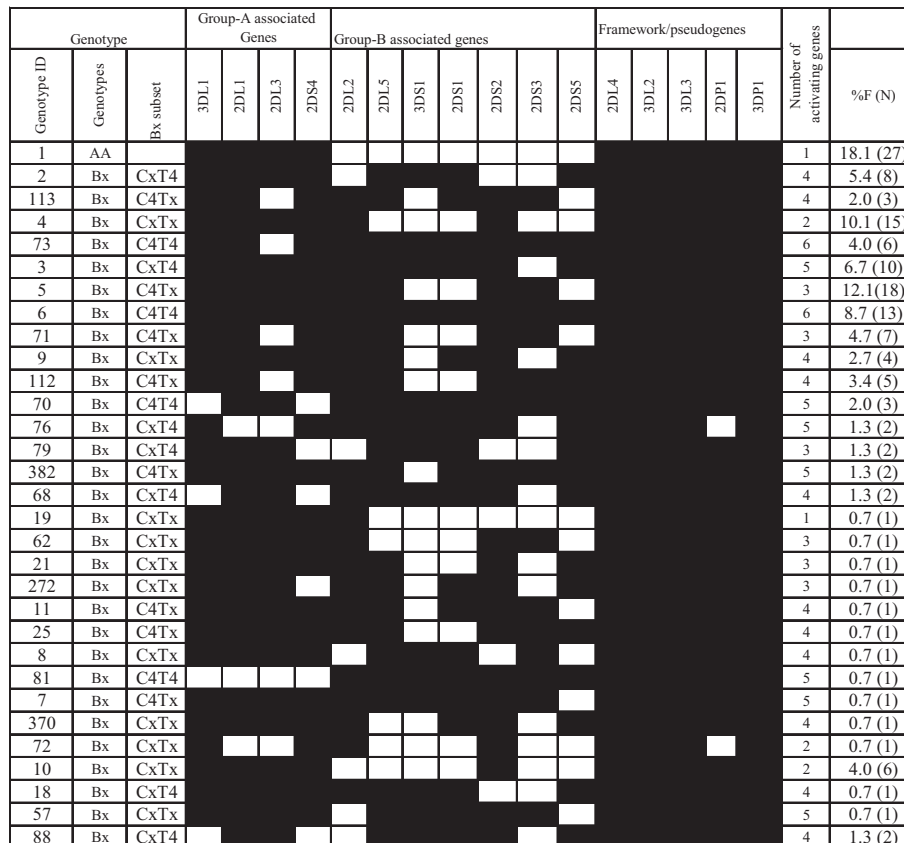


Fig. 1. Presents the KIR gene diversity in our study population. This figure includes the genotype IDs, A and Bx haplotype-associated groups, framework/pseudogenes and the activating KIR gene frequencies. Black areas indicate positive genes, and blank areas indicate negative ones.

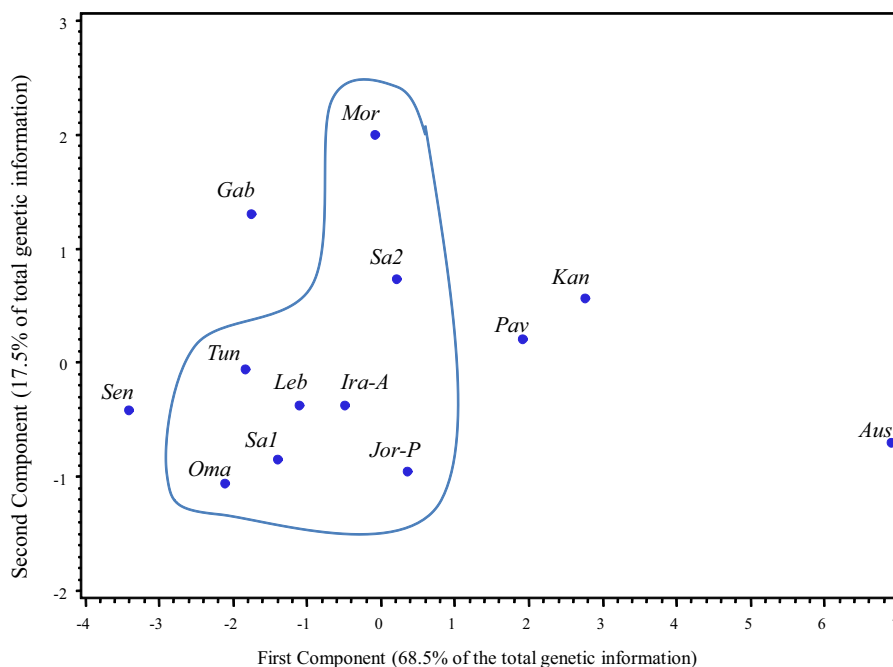


Fig. 2. PCA of nine variable KIR genes (2DL1, 2DL2, 2DL3, 2DS1, 2DS2, 2DS3, 2DS4, 3DL1 and 3DS1) that were commonly reported or typed across all of the populations compared in this study, while we excluded other genes that were not reported in certain studies. Saudis-1 (*Sa1*), Saudis 2 (*Sa2*), Palestinian Jordanians (*Jor-P*), Lebanese (*Leb*), Omanis (*Oma*), Moroccan Chaouya (*Mor*), Tunisians (*Tun*), Iranian Arab (*Ira-A*), Indian Paravar (*Pav*), Indian Kanikar (*Kan*), Gabonese (*Gab*), Senegalese (*Sen*) and Australian Aborigines (*Aus*).

3.2. KIR genotype frequencies

KIR genotypes are a set of KIR genes found in one person and are considered to be more informative markers for differentiating human ethnic groups than the gene frequencies [21]. As indicated in Fig. 1, a total of thirty-one different KIR genotype groups were observed. The most prevalent were 3, 6, 4, 5 and 1, in accord with what has previously been reported [1]. Published reports indicate that lower frequencies of these genotypes are observed among North Asian Indian populations, such as Kanikar and Paravar (2.9%–5.5%), while higher frequencies are found in Japanese and Chinese Han populations (55.2%–58.7%) [5]. Genotype 6, which includes all of the inhibitory and activating KIR genes, was observed at a frequency of 7.9% in this study and at frequencies of 2.8%–5.9% among populations from Iran and Palestine [5,7]. All subsets of Bx genotype gene clusters C4T4, C4Tx, CxT4 and CxTx were observed at frequencies of 19%–31.4% in our study. An absence of one or two of these genotype subsets has been reported in some other populations; for instance, C4Cx, which is observed at frequencies of 40.5% among Iranian Azeri and 31.6% among Iranian Arab, was not detected among American Natives [5].

3.3. Principal components analysis (PCA)

In consideration of the limitations of various typing methods as a reason for unexpected groupings [2], the Arab populations are clustered approximately in the middle between Africans and Asians, as illustrated in Fig. 2. This may support the out-of-Africa theory of migration, which provides some historical perspective regarding the genetic relationships between Arabs and both Africans and Asians. It can also be noted in Fig. 2 that the spatial distance of Australian Aborigines from the Arab populations suggests an absence of any conceivable genetic link. It is worth noting that our HLA data (not yet published) revealed close affinities among Saudi, Omani, Tunisian, Palestinian (Gaza) and Moroccan populations.

In conclusion, this study was able to characterize and describe the KIR gene distributions among healthy, non-blood-related Saudis. These results were consistent with those found in other neighboring populations, but they also reflected disparities compared to other non-Arab populations, such as Australian Aborigines. These findings can be used as a reference control in future studies evaluating the functional significance of the KIR genes and their associations with specific diseases.

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References

- [1] Middleton D, Gonzelez F. The extensive polymorphism of KIR genes. *Immunology* 2010;129:8–19.
- [2] Petlichkovski A, Djulejic E, Trajkov D, Efinska-Mladenovska O, Hristomanova S, Kirijas M, et al. Distribution of killer cell immunoglobulin-like receptor genes in Roma from republic of Macedonia. *Int J Immunogenetics* 2011;38:493–500.
- [3] Shi L, Shi L, Tao Y, Lin K, Liu S, Yu L, et al. Distribution of killer cell immunoglobulin-like receptor genes and combinations with HLA-C ligands in an isolated Han population in southwest China. *Tissue Antigen* 2011;78:60–4.
- [4] Yindom LM, Forbes R, Aka P, Janha O, Jeffries D, Jallow M, et al. Killer-cell immunoglobulin-like receptors and malaria caused by *Plasmodium falciparum* in the Gambia. *Tissue Antigen* 2012;79:104–13.
- [5] Ashouri E, Farjadian S, Reed FE, Ghaderi A, Rajalingam R. KIR gene content diversity in four Iranian populations. *Immunogenetics* 2009;61:483–92.
- [6] Gaafer A, Sheeren A, Iqneibi A, Mohamed G, Al Sulaiman A, Turpeinen H, et al. Killer cell immunoglobulin-like receptor gene diversity in the Saudi population. *Mol Biol Rep* 2011;38(4):2603–10.
- [7] Rayes R, Bazarbachi A, Khazen G, Sabbagh A, Zaatari G, Mahfouz R. Natural killer cell immunoglobulin-like receptors (KIR) genotypes in two Arab populations: will KIR become a genetic landmark between nations. *Mol Biol Rep* 2008;35:225–9.

- [8] Mahfouz R, Rayes R, Mahfoud Z, Bazarbachi A, Zaatari G. Distribution of killer immunoglobulin-like receptors genotypes in the Lebanese population. *Tissue Antigen* 2006;68:66–71.
- [9] Wilson MJ, Torkar M, Haude A, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci USA* 2000;97:4778–83.
- [10] Jamil K, Khakoo S. KIR/HLA interactions and pathogen immunity. *J Biomed Biotechnol* 2011. <http://dx.doi.org/10.1155/2011/298348>.
- [11] Rajalingam R, Du Z, Meenagh A, Luo L, Kavitha J, et al. 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* 2008;60:207–17.
- [12] Martin AM, Freitas EM, Witt CS, Christiansen FT. The genomic organization and evolution of the natural killer immunoglobulin-like receptor (KIR) gene cluster. *Immunogenetics* 2000;51:268–80.
- [13] Denis L, Sivula J, Gourraud P, Kerdudou N, Chout R, et al. Genetic diversity of KIR natural killer cell markers in populations from France, Guadeloupe, Finland, Senegal and Réunion. *Tissue Antigens* 2005;66:267–76.
- [14] Williams F, Middleton D, Leheny W. HLA-A and -B alleles, cytokine polymorphisms and KIR gene frequencies in a population from Oman. *Hum Immunol* 2004;65:1034–8.
- [15] Zhi D, Sun C, Sedimbi KS, Luo F, Shen S, Sanjeevi BC. Killer cell immunoglobulin-like receptor along with HLA-C ligand genes are associated with type1 diabetes in Chinese Han population. *Diabetes Metab Res Rev* 2011;27:872–7.
- [16] McQueen K, Dorighi K, Guethlein L, Wong R, Sanjanwala B, Parham P. Donor-recipient combinations of Group A and B KIR haplotypes and HLA class I ligand affect the outcome of HLA-matched, sibling donor hematopoietic cell transplantation. *Hum Immunol* 2007;68:309–23.
- [17] Abu-Amero K, González A, Larruga AM, Bosley M, Cabrera V. Eurasian and African mitochondrial DNA influences in the Saudi Arabian population. *BMC Evol Biol* 2007;7:32. <http://dx.doi.org/10.1186/1471-2148/7/32>.
- [18] Petraglia M, Alsharekh A. The middle palaeolithic of Arabia, implications for modern human origins, behavior and dispersals. *Antiquity* 2003;77:671–84.
- [19] Gonzalez-Galarza FF, Christmas S, Middleton D, Jones AR. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Res* 2011;39:D913–9.
- [20] *Multivariate Data Reduction and Discrimination with SAS Software* by Ravindra Khattree, Dayanand N. Naik SAS Institute inc. Cary NC, 2000.
- [21] Augusto D, Piovezan B, Tsuneto L, Callegari-Jacques S, Petzl-Erler. KIR gene content in amerindians indicates influence of demographic factors. *PLOS One* 2013;7:E56755.