Distribution of Killer Cell Immunoglobulin-Like Receptor Genes in Albanians from Republic of Macedonia

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Abstract

AIM: The aim of this study was to analyze Killer Ig-Like Receptor (KIR) gene polymorphisms in Albanians from Republic of Macedonia.

MATERIAL AND METHODS: The studied sample consists of 104 healthy unrelated individuals, aged 20-45 years. All individuals are of Albanian nationality, residents of different geographical regions (Skopje, Gostivar, and Tetovo) in Republic of Macedonia. The population genetics analysis package, Arlequin, was used for analysis of the data.

RESULTS: All 16 KIR genes known were observed in the Albanian individuals and framework genes (KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2) were present in all individuals. The frequencies of other KIR genes were: KIR2DP1 (0.981), KIR2DL1 (1), KIR2DL2 (0.615), KIR2DL3 (0.865), KIR2DL5 (0.414), KIR3DL1 (0.933), KIR2DS1 (0.462), KIR2DS2 (0.606), KIR2DS3 (0.327), KIR2DS4 (0.875), KIR2DS5 (0.298), and KIR3DS1 (0.442). Tested linkage disequilibrium (LD) among KIR genes demonstrated that KIR genes present a wide range of linkage disequilibrium.

CONCLUSION: This is the first study analyzing the polymorphism of KIR genes and genotype frequencies in Albanian individuals in the world. The results can be used for anthropological comparisons.

Introduction

The Albanians are an ethnic group inhabiting the Western Balkans that encompasses Republic of Albania and the neighbouring countries. The ancestors of the modern Albanians are considered to be the Illyrians, but admixtures with other ancient populations (for example Thracians) from the Balkans might have occurred [1]. The current size of the entire Albanian population in Europe is estimated to be around 7 million, majority of which live in the Republic of Albania (2,690,000 people) [2] and Kosovo (1,680,000 people) [3]. Significant Albanian populations are found in other neighbouring countries (Serbia, Montenegro, Greece, Macedonia), and there is also Albanian diaspora in some European countries, such as Italy, Germany and Switzerland.

According to the last official census from 2002, in the Republic of Macedonia, Albanians are the largest minority represented with 25.17% of the total population or 509,083 inhabitants [4].

Genetic investigations in Albanian population so far have analyzed the HLA genes distribution [5, 6], Y chromosome polymorphisms [7-10] and prevalence of some genetic disorders [11-13].

Killer cell immunoglobulin-like receptors (KIR) are surface molecules found on subsets of lymphoid cells. Most importantly, they influence the natural killer (NK) cells activity in activating or inhibiting manner, depending on the interaction of KIR with HLA molecules present on the target host cells [14, 15]. The **KIR** locus contains a family of polymorphic and
highly homologous members (14 genes and 2 pseudogenes), which can be activating or inhibitory. Based on the gene content, the haplotypes have been resolved into two broad sets, termed A and B [16]. The different KIR haplotypes vary in the number and type of genes present, but the genes KIR3DL3, KIR3DP1, KIR2DL4 and KIR3DL2 are present on virtually all haplotypes and have therefore been termed framework genes [17]. Population studies performed over the last two decades have revealed extensive diversity at the KIR gene locus, which derives from both, its polygenic and multi-allelic polymorphism, whereas on the basis of gene content, haplotype B displays a much greater variety of subtypes [18, 19].

The aim of this study was to examine KIR gene polymorphisms by determining the frequencies of 16 KIR genes and pseudogenes (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DP1, and KIR3DP1) and KIR genotypes in Albanians from the Republic of Macedonia.

To our knowledge, this is the first study of the diversity of KIR genes in Albanian individuals, both from the Republic of Macedonia and in the world.

Material and Methods

Population samples

The study included 104 unrelated healthy Albanian individuals (Macedonian Albanians), residents of different regions of the Republic of Macedonia (Gostivar, Skopje, Tetovo). Each individual was interviewed on a one-to-one basis; his/her genealogy was recorded for the last three generations. Admixture, if any, was recorded for each individual. Individuals with only one Albanian parent were excluded from the study. After signing of written consent, genomic DNA was extracted from the peripheral blood leukocytes using standard phenol/chloroform procedure, described elsewhere [20], and stored in the anthropology project field of the Macedonian Human DNA Bank (hDNAMKD) [21] until processing.

PCR amplification

For KIR genotyping, commercially available PEL-FREEZ KIR genotyping SSP kit (Dynal Biotech, Brown Deer, WI) was used. It is a PCR-based method (using sequence-specific priming approach) designed to detect the presence or absence of 16 KIR genes and pseudogenes defined by the International nomenclature committee of WHO [22, 23]. In brief, locus specific primer sets, dispensed in a 96 well thermal tray were used for amplification of genomic DNA. After the amplification, the PCR products are loaded and separated by electrophoresis onto a 2% agarose gel stained with ethidium bromide, after which the results are interpreted using a worksheet for the specific amplification patterns. The presence of each KIR gene was determined by the presence of a band of DNA of the expected size.

All PCRs contained an internal positive control consisting of an additional pair of primers specific for the growth hormone (GH) gene and a negative control [24]. Individuals were determined negative for a particular KIR gene when a band of expected size was absent in the presence of a band for the GH gene. We have used external quality control consisting of cell lines from Immunogenetics and Histocompatibility Worshop Conferences and Centre d’ Etude du Polymorphisme Humain.

Statistical analysis

The occurrence of KIR genes in individuals (frequency = F) was obtained by direct counting. Gene

### Table 1: Comparison of the observed and estimated KIR gene frequencies for Macedonian Albanians (N = 104) and Native Macedonians (N=214).  

| Gene | KIR2DP1 | KIR3DP1 | KIR2DL1 | KIR2DL2 | KIR2DL3 | KIR2DL4 | KIR2DL5 | KIR3DL1 | KIR3DL2 | KIR3DL3 | KIR2DS1 | KIR2DS2 | KIR2DS3 | KIR2DS4 | KIR2DS5 | KIR3DS1 | KIR2DP1 | KIR3DP1 |
|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Macedonian Albanians (N) | 102 | 104 | 104 | 64 | 89 | 104 | 43 | 97 | 104 | 104 | 48 | 63 | 34 | 91 | 31 | 46 |
| Macedonian Albanians (P) | 0.961 | 1 | 1 | 0.615 | 0.865 | 1 | 0.414 | 0.933 | 1 | 1 | 0.462 | 0.606 | 0.327 | 0.875 | 0.298 | 0.442 |
| Macedonian Albanians (GF) | 0.862 | 1 | 1 | 0.380 | 0.633 | 1 | 0.234 | 0.741 | 1 | 1 | 0.267 | 0.372 | 0.180 | 0.646 | 0.162 | 0.253 |
| Macedonians (N) | 210 | 214 | 201 | 126 | 192 | 214 | 89 | 201 | 214 | 214 | 103 | 122 | 77 | 201 | 64 | 84 |
| Macedonians (P) | 0.980 | 1 | 0.940 | 0.590 | 0.897 | 1 | 0.415 | 0.945 | 1 | 1 | 0.481 | 0.570 | 0.360 | 0.945 | 0.300 | 0.392 |
| Macedonians (GF) | 0.870 | 1 | 0.760 | 0.360 | 0.690 | 1 | 0.230 | 0.800 | 1 | 1 | 0.290 | 0.350 | 0.180 | 0.800 | 0.170 | 0.220 |
| Pearson’s χ² | 1 & 0.001 | 0.650 & 0.280 | & 0.967 & 0.821 | & 0.740 | 0.545 | 0.564 | 0.059 | 0.996 | 0.397 |
| OR | 1.029 & & 1.118 & 0.680 & & 0.995 & 0.896 & & 0.924 & 1.159 & 0.864 & 0.453 & 0.995 & 1.227 |

Wald 95% CI | 0.185- & 0.692- & 0.337- & 0.615- & 0.547- & 0.176- & 0.578- & 0.719- & 0.526- & 0.202- & 0.596- & 0.764- |

N, number of individuals; F, observed frequency was obtained by direct counting; GF, gene frequencies were calculated using the formula GF=1-(1-F); p, statistical significance; χ², cannot be calculated because expected <5, χ² test; OR; Odds ratio; CI, confidence interval.
Table 2: LD analysis for KIR loci for Macedonian Albanians (N = 104) and Macedonians (N = 214).

| Gene Combination | D' | P     | Gene Combination | D' | P     |
|------------------|----|-------|------------------|----|-------|
| KIR3DS1 KIR3DL1 | 1.0000 | 0.0000 | KIR3DL3 KIR3DL4 | 0.0000 | 0.0000 |
| KIR3DS1 KIR3DL2 | 0.0000 | 0.0000 | KIR3DL3 KIR2DL2 | 0.0000 | 0.0000 |
| KIR3DL1 KIR2DL2 | 0.0000 | 0.0000 | KIR3DL3 KIR2DL3 | 0.0000 | 0.0000 |
| KIR3DL1 KIR2DL3 | 0.0000 | 0.0000 | KIR3DL1 KIR2DL4 | 0.0000 | 0.0000 |
| KIR3DL1 KIR3DL2 | 0.0000 | 0.0000 | KIR3DL3 KIR2DL4 | 0.0000 | 0.0000 |
| KIR3DL1 KIR2DL4 | 0.0000 | 0.0000 | KIR3DL1 KIR3DL2 | 0.0000 | 0.0000 |

LD values for two KIR genes were calculated using the formula:

\[ D' = \frac{P_{xy} - P_{x}P_{y}}{\sqrt{P_{x}(1-P_{x})P_{y}(1-P_{y})}} \]

where \(D'\) is the Linkage Disequilibrium coefficient, \(P_{x}\) and \(P_{y}\) are the observed and expected allele frequencies, respectively.

**Results**

KIR gene frequencies

The presence and absence of the 16 KIR genes (14 genes and 2 pseudogenes) determined in the 104 healthy individuals, randomly selected from Macedonian population, were used for comparison with KIR gene frequencies of the studied Albanian population.
the Macedonian Albanians is shown in Table 1, along with the corresponding frequencies for the KIR genes in the native Macedonians [29]. All 16 KIR genes were observed in the Macedonian Albanians and framework genes (KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2) were present in all individuals. The frequencies of other KIR genes were: KIR2DP1 (0.981), KIR2DL1 (1), KIR2DL2 (0.615), KIR2DL3 (0.865), KIR2DL5 (0.414), KIR1DL1 (0.933), KIR2DS1 (0.462), KIR2DS2 (0.606), KIR2DS3 (0.327), KIR2DS4 (0.875), KIR2DS5 (0.298), and KIR3DS1 (0.442). The corresponding calculated gene frequencies were: KIR2DP1 (0.862), KIR2DL1 (1), KIR2DL2 (0.380), KIR2DL3 (0.633), KIR2DL5 (0.234), KIR3DL1 (0.741), KIR2DS1 (0.267), KIR2DS2 (0.372), KIR2DS3 (0.180), KIR2DS4 (0.646), KIR2DS5 (0.162), and KIR3DS1 (0.253).

Comparison of KIR gene frequencies between Macedonian Albanians and Macedonians reveals statistically significant differences for KIR2DL1 (p = 0.001) and KIR2DS4 (p = 0.050) (Table 1).

**Linkage Disequilibrium**

The classical linkage disequilibrium coefficient (\(D\)), linkage disequilibrium coefficient \(D\) standardized by the maximum value it can take (\(D_{\text{max}}\)), given the allele frequencies (\(D\)), standardised simple measure of linkage disequilibrium (\(r^2\)), and statistical significance (\(P\)) for KIR genes are shown in Table 2. The genes present in all individuals (KIR3DP1, KIR2DL4, KIR3DL2 and KIR3DL3) were excluded from the analysis.

Pairs of KIR loci that displayed the most significant (\(P < 0.05\)) LD in Macedonian Albanians are given in Table 3. The most striking positive LD (\(P < 0.0001\)) was observed between pairs KIR3DL1 and KIR2DS4, KIR2DL1 and KIR3DL1, KIR2DS4 with KIR3DL1 and KIR2DL1, KIR2DL2 with KIR2DS2 and KIR2DS3. Furthermore, KIR2DL5 was in positive LD with KIR2DL2 and several activating genes (KIR3DS1, KIR2DS1, KIR2DS3, and KIR2DS5), very similar to KIR3DS1 which was in highly significant positive LD with KIR2DL5, KIR2DS1, KIR2DS3, and KIR2DS5. This pattern of highly significant LD within several pairs of activating KIR genes was also observed for KIR2DS1, KIR2DS3 and KIR2DS5. As for negative LD, the highest significance (\(P < 0.0001\)) was observed between pairs KIR2DL2 and KIR2DL3, KIR2DS2 and KIR2DL2, KIR2DS5 with KIR3DL1 and KIR2DS4, and also for KIR2DP1 and KIR2DS2.

**Genotype frequencies**

KIR groups, genotype ID, KIR genotypes, number of individuals displaying certain genotype, and the frequency of genotypes are given in Table 4.

If any of the genes 2DL2, 2DL5, 3DS1, 2DS1, 2DS2, 2DS3, or 2DS5 was present, the genotype was considered as B. If none of these were present, genotype is considered as AA. We have not attempted to distinguish between AB and BB genotypes and called any of this Bx. KIR genotypes were numerated according to the Allele frequencies KIR Database [30, 31]. Total of 45 different KIR genotypes were found to be present in studied population, based on the presence of 16 KIR genes. We have found two group AA genotype (genotype ID 1 and 180) with frequency of 0.192 and 0.010 respectively, and 43 group Bx genotypes. The most frequent genotypes in the Bx group were genotypes ID 4 with frequency of 0.164, and ID 2 with frequency of 0.067. Five new genotypes of the Bx group were found (Table 4).

There is not statistically significant difference in distribution of AA and Bx KIR genotypes between Macedonian Albanians and native Macedonians (\(P = 0.789\), OR = 0.924, Wald 95% CI = 0.518-1.649) (Table 5).

**Discussion**

We present for the first time KIR genes distribution in Albanian individuals living in the Republic of Macedonia. Albanians have probably
Table 4: KIR locus haplogroups, genotypes ID and genotype frequency of Macedonian Albanians (N = 104) and Macedonians (N = 214).

| KIR Genotype ID | Macedonian Albanians No (%) | Macedonians No (%) | Pearson’s P |
|----------------|----------------------------|--------------------|-------------|
| KIR 3DL1 | 1 | 100 | 1 | 100 | 1.000 | 0.998 |
| KIR 2DL1 | 2 | 100 | 2 | 100 | 1.000 | 0.998 |
| KIR 2DS4 | 3 | 100 | 3 | 100 | 1.000 | 0.998 |
| KIR 3DS1 | 4 | 100 | 4 | 100 | 1.000 | 0.998 |
| KIR 2DS2 | 5 | 100 | 5 | 100 | 1.000 | 0.998 |
| KIR 2DS9 | 6 | 100 | 6 | 100 | 1.000 | 0.998 |
| KIR 3DS3 | 7 | 100 | 7 | 100 | 1.000 | 0.998 |
| KIR 3DL2 | 8 | 100 | 8 | 100 | 1.000 | 0.998 |
| KIR 3DS1 | 9 | 100 | 9 | 100 | 1.000 | 0.998 |
| KIR 2DL2 | 10 | 100 | 10 | 100 | 1.000 | 0.998 |
| KIR 3DL1 | 11 | 100 | 11 | 100 | 1.000 | 0.998 |
| KIR 3DS2 | 12 | 100 | 12 | 100 | 1.000 | 0.998 |
| KIR 2DS2 | 13 | 100 | 13 | 100 | 1.000 | 0.998 |
| KIR 2DL1 | 14 | 100 | 14 | 100 | 1.000 | 0.998 |
| KIR 3DS1 | 15 | 100 | 15 | 100 | 1.000 | 0.998 |
| KIR 3DS2 | 16 | 100 | 16 | 100 | 1.000 | 0.998 |
| KIR 2DS2 | 17 | 100 | 17 | 100 | 1.000 | 0.998 |
| KIR 3DS2 | 18 | 100 | 18 | 100 | 1.000 | 0.998 |
| KIR 2DL2 | 19 | 100 | 19 | 100 | 1.000 | 0.998 |
| KIR 3DL2 | 20 | 100 | 20 | 100 | 1.000 | 0.998 |
| KIR 3DS1 | 21 | 100 | 21 | 100 | 1.000 | 0.998 |
| KIR 2DL1 | 22 | 100 | 22 | 100 | 1.000 | 0.998 |
| KIR 3DS2 | 23 | 100 | 23 | 100 | 1.000 | 0.998 |
| KIR 2DS2 | 24 | 100 | 24 | 100 | 1.000 | 0.998 |
| KIR 3DS2 | 25 | 100 | 25 | 100 | 1.000 | 0.998 |
| KIR 2DL2 | 26 | 100 | 26 | 100 | 1.000 | 0.998 |
| KIR 3DL2 | 27 | 100 | 27 | 100 | 1.000 | 0.998 |
| KIR 3DS1 | 28 | 100 | 28 | 100 | 1.000 | 0.998 |
| KIR 2DL1 | 29 | 100 | 29 | 100 | 1.000 | 0.998 |
| KIR 3DS2 | 30 | 100 | 30 | 100 | 1.000 | 0.998 |

*Populated the southern-west part of the Balkan in the Palaeolithic Age. During the various invasions and migrations that frequently occurred in these territories, genetic admixtures within Roman and later Byzantine empires probably took place.*
When compared with the KIR gene frequencies of the native Macedonian population, the most significant difference was found for KIR2DL1 (F = 1 in Macedonian Albanians and F = 0.940 in Macedonians, P = 0.001) and for KIR2DS4 (0.875 compared to 0.940 in Macedonian population, P = 0.050). As expected, the frequencies of several KIR genes are comparable or even very similar with frequencies found in some Mediterranean populations, such as France, Italy, Greece, and Belgium [32-35]. Relatively high frequency of KIR2DS4 (0.875) is common for both, Macedonian Albanians and native Macedonians and it is similar to the frequencies found in populations from Belgium, England, Greece and Japan [35-37]. These findings are in agreement with previously published reports studying the HLA polymorphisms, which indicate similarity of Albanians with other Balkan and European (especially Mediterranean) ethnic groups [6].

| Haplogroup | Albanians (N = 104) | Macedonians (N = 214) | Pearson’s p-value | Odds ratio | Wald 95% CI |
|------------|---------------------|-----------------------|-------------------|------------|-------------|
| AA         | 21                  | 23                    | 0.302             | 0.215      | 0.789       | 0.924       | 0.518-1.649 |
| Bx         | 83                  | 168                   | 0.798             |            |             |             |             |

N. number of individuals displaying AA or Bx KIR genotype; F. frequency of KIR genotype; Cl. confidence interval.

Linkage disequilibrium (LD) analysis is used in KIR population studies in order to define common co-segregation patterns of multiple KIR loci and also potential allelic relationships between KIR loci. We have observed several pairs in striking positive LD relationship (P < 0.0001) in our study, such as pairs KIR3DL1 and KIR2DS4, KIR2DL1 and KIR3DL1, and combinations between KIR2DL5 and several activating KIR3DS1, KIR2DS1, KIR2DS3 and KIR2DS5.

Most of the observed LD patterns have been previously reported for Macedonian population (29), and Roma population from the Republic of Macedonia [38] and also other populations [39]. However, we cannot assume an absolute correlation between the KIR loci, as we only detect a certain percentage of alleles at a locus.

Despite the mentioned differences in the frequencies of two KIR genes in the studied Macedonian Albanians and native Macedonian population, we have not found statistically significant differences between the two populations when comparing the frequencies of AA and Bx KIR genotypes. Similar predominance of group Bx genotypes has been also observed in the North Indians, Palestinians, South Asians and Afro-Caribbean’s [36, 39, 40]. Five new genotypes were identified and are being referred to allelefrequencies.net [30].

We published several papers about frequencies of KIR genes: in human West Nile virus infections reported 2011 in the Republic of Macedonia [41]; in Graft versus Host Disease after related haematopoietic stem cell transplantation in patients with haematological malignancies from Republic of Macedonia [42]; in pandemic influenza A (H1N1)pdm09 infection in critically ill Macedonian patients [43]; in Macedonian patients with haematological malignancies [44]; and women with infertility problems [45]. The frequency of Macedonian Albanians in these and future studies of KIR genes should be taken into account.

There are few limitations of this study that should be addressed. First, KIR typing at allelic level might be more informative but unfortunately, at present we are not able to perform it. Second, the genetic distance analysis between studied and other populations, would certainly add value to this study. However, there are recent studies arguing the real meaning and contribution of this analysis to the population comparisons, in the light of proposed different evolution of activating and inhibitory KIR genes [46, 47].

In conclusion, we have determined the distribution of KIR genes in Macedonian Albanians and compared it with similar results for native Macedonian populations. It would be of interest to compare these results with results for other Albanian populations in the region and throughout the world and later address the influence of migrations and admixture of populations on inheritance of KIR genes. This study can be suitable for use in other anthropological studies in order to better understand genetic distances, and especially for performing a meta-analysis of KIR gene frequencies in Albanians worldwide.

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