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Distribution of killer cell immunoglobulinlike receptors in the Macedonian population

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ABSTRACT

The aim of this study was to analyze killer immunoglobulinlike receptor (KIR) gene polymorphism in the Macedonian population. The study sample consists of 214 healthy unrelated individuals, aged 20–35 years. All individuals are of Macedonian origin and nationality, and residents of different geographic regions. The population genetics analysis package, Arlequin, was used for analysis of the data. We found that all 16 KIR genes were observed in the Macedonian population and framework genes *KIR3DL3*, *KIR2DL4*, and *KIR3DL2* were present in all individuals. A total of 56 different KIR genotypes were found in the Macedonian population, based on the presence of 16 K*IR* genes. Neighbor-joining phylogenetic tree, constructed on the basis of standard genetic distances of KIR genes, shows that Macedonian population is in the same cluster with England West Midlands Indian Asian, Brazil SouthEast Caucasian, Romania Caucasians, Spain Basque, England West Midlands Caucasian, France Reunion, and Spain Granada populations. The frequency of KIR loci in Macedonian population shares several general features with other Caucasoid populations studied before.

1. Introduction

Killer immunoglobulinlike receptors (*KIR*) are cell-surface molecules important in the regulation of activity of the natural killer cells and some T cells in either inhibitory or activating manner [1]. K*IR* molecules are encoded by the K*IR* gene family clustered within the leukocyte receptor complex on chromosome 19q13.4 [2,3]. K*IR* genes exhibit allelic, haplotypic, and gene content variability [4–7]. K*IR* haplotypes differ in the number and type of K*IR* genes they contain. Thus, K*IR* haplotypes can be distinguished according to their gene content. Because the individual K*IR* genes are polymorphic, K*IR* haplotypes that are identical by gene content can be further subdivided according to allele combination. Diversity in human K*IR* genotype therefore is sum from three components: haplotypic gene content, allelic polymorphism, and the combination of maternal and paternal haplotypes. The combined effects of these three components are such that unrelated individuals usually differ in K*IR* genotype and ethnic populations have widely differing K*IR* genotype frequencies.

In general, two KIR haplotypes are recognized: A and B [7–9]. The latest haplotype definition (14th International HLA and Immunogenetics Workshop, 2005) identifies haplotype A to be composed of *KIR3DL3*, *KIR2DL3*, *KIR2DP1*, *KIR2DL1*, *KIR3DP1*, *KIR2DL4*, *KIR3DL1*, *KIR2DS4*, and *KIR3DL2* genes, whereas all other haplotypes are described as haplotype B.

With the exception of null alleles and possibly K*IR 3DL3*, all known K*IRs* in a given individual's K*IR* gene repertoire are expressed [1–12]. In heterozygous individuals, natural killer cell clones can express none, one or both alleles of K*IR 3DL1* and K*IR 3DL2*, but usually both alleles of K*IR 2DL4* [13].

Population studies have revealed that KIR gene frequencies and genotype content vary considerably among different ethnic groups, and that frequencies of A and B haplotypes have also been found to differ between populations [4,7,14–29]. Results from population studies till date indicate the extent of KIR gene diversity; some of these studies have also shown the association of the presence or absence of specific KIR genes in certain human diseases [30–34].

Although comparisons with the major histocompatibility complex are obvious, what is less clear are the functional implications of KIR diversity and whether it serves us a useful purpose [1,35].

The Macedonian population is of special interest for anthropological studying in the light of open questions regarding its origin and the migrations, which resulted in settlement of Macedonians in the heart of Balkan Peninsula. Macedonia is located in the Central Balkans, bordering Bulgaria, Greece, Albania, Serbia and Kosovo province, covering an area of 25,710 km. According to the 2002 census, the country's population was 2,022,577. Data on the declared ethnic affiliation from the 2002 census reported that 64.1% of the population identify themselves as Macedonian, 25.17% as Alba-

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nian, 3.95% as Turk, 2.66% as Roma, 1.78% as Serb, 0.84% as Bosniac, 0.48% as Vlach, and 1.04% as others [36]. The country seceded peacefully from Yugoslavia after an independence referendum, held in September 1991.

This is the first study of the diversity of KIR genes in Macedonian populations. The aim of this study was to determine 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*), genotypes, and to compare them with populations for which similar data are available. Finally, genetic distances between Macedonian and other compared populations were calculated.

2. Subjects and methods

2.1. Population samples

The study included 214 unrelated healthy individuals, all Macedonians of Macedonian origin and nationality, and residents of different regions of the Republic of Macedonia. Each individual was interviewed on a one-to-one basis, his/her genealogy was recorded for the last three generations, and a signed consent was obtained. Admixture, if any, was recorded for each individual. Individuals with only one Macedonian parent were excluded from the study. After signing of written consent, genomic DNA was extracted from the peripheral blood leukocytes using standard phenol or chloroform procedure, described elsewhere [37], and stored in the anthropology project field of the Macedonian Human DNA Bank (hDNAMKD) [38] until processing.

2.2. Polymerase chain reaction amplification

For KIR genotyping, commercially available PEL-FREEZ KIR genotyping SSP kit (Dynal Biotech, Brown Deer, WI) was used. It is a polymerase chain reaction (PCR)–based method (using sequence-specific priming approach) designed to detect the presence and/or absence of 16 KIR genes and pseudogenes defined by the International nomenclature committee of World Health Organization [39,40]. Briefly, 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 gene [41], and a negative control. 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.

2.3. Statistical analysis

The occurrence of KIR genes in individuals (f) was obtained by direct counting. Gene frequencies were calculated using the formula $F = 1-\sqrt{(1\ \mathrm{F})}$. For analysis of the molecular polymorphism of the locus studied, the Arlequin software version 3.0 [42] (Genetics and Biometry Laboratory, University of Geneva, Switzerland) was used. The Hardy–Weinberg equilibrium [43] and the Ewans–Watterson [44] statistics were calculated to examine the presence of selective forces influencing the allele distribution. The percentage of individuals carrying each KIR gene was also calculated. Linkage disequilibrium (LD) values for two locus associations were calculated using 2×2 tables [45]. Because LD is not independent of allele frequencies, normalized LD was calculated as described previously [46,47]. The statistical significance of LD values was assessed by Yates $\times$ two analysis on the corresponding 2×2 contingency table. A neighbor-joining tree was constructed from Nei's genetic distances using the PHYLIP phylogeny inference package [48].

Group nomenclature (AA, AB, BB) of KIR was defined if any of genes *KIR2DL2*, *KIR2DL5*, *KIR3DS1*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5* are present, the genotype is taken as having B. If none of these are present genotype is considered as AA.

If any are present and *KIR3DL1*, *KIR2DL1*, *KIR2DL3*, and *KIR2DS4* are all present then genotype is considered as AB. However, if any of *KIR3DL1*, *KIR2DL1*, *KIR2DL3*, *KIR2DS4* genes are missing, genotype is considered as BB. KIR genotypes were numerated according to the Allelefrequencies KIR Database [23,49].

For comparison of KIR gene frequencies and genetic distance analysis, we have used data for KIR genotypic polymorphisms published at the Allele Frequencies database (http://www.allelefrequencies.net) [44]. The number of KIR genes allocated at this database differs for different populations, depending on the typing method used. We have included in our study and used for comparison only those having complete data for 14 different genes. According to this criterion, 33 populations were selected out of total of 82 populations.

3. Results

3.1. KIR gene frequencies

The presence and absence of the 16 KIR genes (14 genes and 2 pseudogenes) determined in the 214 healthy individuals, randomly selected from the Macedonian population are shown in Table 1. All 16 KIR genes were observed in the Macedonian population and framework genes *KIR3DL3*, *KIR2DL4*, and KIR3DL2 were present in all individuals. The most frequently present KIR genes were *KIR2DL1* (94%), *KIR2DL2* (59%), *KIR2DL3* (89%), *KIR2DL5* (41%), *KIR3DL1* (93%), *KIR2DP1* (98%), *KIR2DS1* (48%), *KIR2DS2* (56%), *KIR2DS3* (36%), *KIR2DS4* (94%), *KIR2DS5* (30%), *KIR3DS1* (39%), and *KIR3DP1* (99%). Most of the individuals (99.5%) had *KIR3DP1*, 99% had KIR3DP1*003, and 24% had KIR3DP1*001/002. In 41% of individuals we found *KIR2DL5*; 28% had KIR2DL5A*001 and 25% had KIR2DL5B*002/004. In 94% of individuals we found *KIR2DS4*; 25% had KIR2DS4*001-002 and 85.9% had KIR2DS4*003 (Table 1).

3.2. Genotype frequencies

KIR genotypes, groups, genotype ID, number of individuals, and percentage of distribution are given in Figure 1. A total of 56

Table 1
Observed and estimated KIR gene frequencies for a Macedonian population ($N = 214$)

	Frequencies for Macedonian population															
	Pseudogenes		Inhibitory KIR								Noninhibitory KIR					
	KIR 2DP1	*KIR 3DP1*	*KIR 2DL1*	*KIR 2DL2*	*KIR 2DL3*	*KIR 2DL4*	*KIR 2DL5*	*KIR 3DL1*	*KIR 3DL2*	*KIR 3DL3*	*KIR 2DS1*	*KIR 2DS2*	*KIR 2DS3*	*KIR 2DS4*	*KIR 2DS5*	*KIR 3DS1*
OF	0.98	0.995	0.949	0.589	0.897	1	0.416	0.939	1	1	0.481	0.565	0.360	0.944	0.304	0.392
S.D. of OF	0.009	0.005	0.015	0.034	0.021	0	0.034	0.016	0	0	0.034	0.034	0.033	0.16	0.031	0.033
EF	0.86	0.90	0.76	0.36	0.67	1	0.24	0.74	1	1	0.28	0.34	0.20	0.76	0.17	0.22

OF, observed frequency was obtained by direct counting; S.D. of OF, standard deviation of observed frequency; EF, estimated frequencies were calculated using the formula EF = $1-\sqrt{(1-F)}$.