ID Design 2012/DOOEL Skopje
South East European Journal of Human Genetics
Volume 2015; Article ID 40001, 8 pages
http://dx.doi.org/10.3889/seejhg.2015.40001
Basic Human Genetics

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

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Citation: Petlichkovski A, Djulejic E, Trajkov D, Efinska-Mladenovska O, Hristomanova Mitkovska S, Spiroski M. Distribution of Killer Cell Immunoglobulin-Like Receptor Genes in Albanians from Republic of Macedonia. SEE J Hum Genet. 2015 Feb 19; 2015:40001. http://dx.doi.org/10.3889/seejhg.2015.40001

Key words: Killer immunoglobulin-like receptor (KIR) gene polymorphism; KIR genotyping; PCR-SSP; Albanians from Macedonia.

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Received: 28-Nov-2014; **Revised:** 29-Dec-2014; **Accepted:** 30-Jan-2015; **Published:** 19-Feb-2015

Competing Interests: The author have declared that no competing interests exist.

Abstract

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

MATERIAL NA 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

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

	Frequencies for *KIR* of Macedonian Albanians and native Macedonians															
	Pseudogenes		Inhibitory *KIR*								Non inhibitory *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*
Macedonian Albanians (N)	102	104	104	64	89	104	43	97	104	104	48	63	34	91	31	46
Macedonian Albanians (F)	0.981	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 (F)	0.980	1	0.940	0.590	0.897	1	0.415	0.940	1	1	0.481	0.570	0.360	0.940	0.300	0.392
Macedonians (GF)	0.870	1	0.760	0.360	0.690	1	0.230	0.800	1	1	0.280	0.350	0.180	0.800	0.170	0.220
Pearson's p	1	&	0.001	0.650	0.280	&	0.967	0.821	&	&	0.740	0.545	0.564	0.050	0.986	0.397
OR	1.029	&	&	1.118	0.680	&	0.990	0.896	&	&	0.924	1.159	0.864	0.453	0.995	1.227
Wald 95% CI	0.185-5.713	&	&	0.692-1.806	0.337-1.373	&	0.615-1.593	0.347-2.318	&	&	0.578-1.477	0.719-1.868	0.526-1.419	0.202-1.015	0.596-1.661	0.764-1.973

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; &, cannot be calculated because expected <5, $\chi 2$ test; OR, Odds ratio; CI, confidence interval.

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 Worskshop 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 2: LD analysis for *KIR* loci for Macedonian Albanians (N = 104) and Macedonians (N = 214).

	Macedonian Albanians (n = 104)											
	KIR3DL1	*KIR2DL1*	*KIR2DL3*	*KIR2DS4*	*KIR2DL2*	*KIR2DL5*	*KIR3DS1*	*KIR2DS1*	*KIR2DS2*	*KIR2DS3*	*KIR2DS5*	*KIR2DP1*
KIR3DL1												
D^a		&	0.0006	0.0589	-0.0163	-0.0395	-0.0375	-0.0362	-0.0169	-0.0261	-0.0376	-0.0013
D’		&	0.0095	1.0000	-0.6286	-1.0000	-1.0000	-1.0000	-0.6376	-0.5755	-0.7965	-1.0000
r^2		&	0.0000	0.5052	0.0178	0.1024	0.0910	0.0842	0.0191	0.0492	0.1078	0.0014
P		&	0.0044	0.0000	0.1734	0.0011	0.0021	0.0031	0.1588	0.0237	0.0008	0.7013
KIR2DL1												
D^a	0.0156		&	&	&	&	&	&	&	&	&	&
D’	0.3225		&	&	&	&	&	&	&	&	&	&
r^2	0.0871		&	&	&	&	&	&	&	&	&	&
P	<0.0001		&	&	&	&	&	&	&	&	&	&
KIR2DL3												
D	0.0031	0.0134		0.0024	-0.0325	-0.0213	-0.0078	-0.0148	-0.0242	-0.0329	0.0113	0.0166
D’	0.0569	0.2907		0.0222	-0.6286	-0.2693	-0.1034	-0.2041	-0.4564	-0.3633	0.2811	1.0000
r^2	0.0018	0.0400		0.0005	0.0384	0.0160	0.0021	0.0076	0.0211	0.0423	0.0052	0.1261
P	0.5318	0.0034		0.8281	0.0456	0.1970	0.6403	0.3753	0.1386	0.0360	0.4612	0.0003
KIR2DS4												
D	0.0480	0.0158	-0.0011		-0.0192	-0.0445	-0.0601	-0.0577	-0.0204	-0.0553	-0.0493	-0.0024
D’	0.9113	0.3258	-0.1894		-0.4000	-0.6066	-0.8621	-0.8571	-0.4146	-0.6571	-0.5616	-1.0000
r^2	0.7628	0.0968	0.0002		0.0143	0.0746	0.1339	0.1224	0.0160	0.1270	0.1061	0.0028
P	<0.0001	<0.0001	0.8192		0.2229	0.0054	0.0002	0.0004	0.1973	0.0003	0.0009	0.5894
KIR2DL2												
D	-0.0110	-0.0165	-0.0423	-0.0090		0.0436	0.0259	0.0237	0.2041	0.0969	0.0089	-0.0074
D’	-0.4388	-0.7789	-1.0000	-0.3920		0.2744	0.1522	0.1333	0.8762	0.7706	0.0774	-1.0000
r^2	0.0087	0.0230	0.0800	0.0064		0.0332	0.0115	0.0095	0.7373	0.1803	0.0016	0.0123
P	0.1725	0.0266	<0.0001	0.2427		0.0632	0.2746	0.3196	<0.0001	<0.0001	0.6842	0.2589
KIR2DL5												
D	-0.0215	-0.0207	-0.0227	-0.0234	0.0822		0.1633	0.1553	0.0380	0.0956	0.1268	-0.0017
D’	-0.6049	-0.6887	-0.3775	-0.7147	0.4808		0.7081	0.6977	0.2331	0.4986	0.7250	-0.1475
r^2	0.0332	0.0361	0.0229	0.0426	0.1150		0.4457	0.4003	0.0249	0.1713	0.3167	0.0006
P	0.0077	0.0054	0.0268	0.0025	<0.0001		<0.0001	<0.0001	0.1073	<0.0001	<0.0001	0.8019
KIR3DS1												
D	-0.0276	-0.0172	-0.0157	-0.0294	0.0399	0.1311		0.2093	0.0205	0.0958	0.1566	-0.0011
D’	-0.7467	-0.5510	-0.2517	-0.8628	0.2473	0.5720		0.8789	0.1177	0.5254	0.9422	-0.1034
r^2	0.0558	0.0255	0.0112	0.0684	0.0276	0.2969		0.7147	0.0072	0.1690	0.4753	0.0003
P	0.0005	0.0196	0.1209	0.0001	0.0151	<0.0001		<0.0001	0.3885	<0.0001	<0.0001	0.8683
KIR2DS1												
D	-0.0315	-0.0173	-0.0113	-0.0291	0.0390	0.1223	0.1522		0.0185	0.0703	0.1605	-0.0007
D’	-1.0000	-0.6495	-0.2113	-1.0000	0.1973	0.5668	0.7475		0.1016	0.3992	1.0000	-0.0714
r^2	0.0697	0.0246	0.0055	0.0640	0.0252	0.2465	0.3891		0.0058	0.0903	0.4954	0.0001
P	0.0001	0.0217	0.2774	0.0002	0.0202	<0.0001	<0.0001		0.4389	0.0022	<0.0001	0.9123
KIR2DS2												
D	-0.0020	-0.0081	-0.0447	0.0316	0.2278	0.0304	-0.0077	0.0198		0.0904	0.0021	-0.0076
D’	-1.0000	-1.0000	-1.0000	0.1511	0.9799	0.1782	-0.2920	0.1504		0.7016	0.0181	-1.0000
r^2	0.0036	0.0146	0.0881	0.0163	0.8725	0.0158	0.0042	0.0076		0.1556	0.0001	0.0128
P	0.3795	0.0767	<0.0001	0.0620	<0.0001	0.0662	0.3409	0.2027		0.0001	0.9227	0.2493
KIR2DS3												
D	-0.0109	0.0091	-0.0424	-0.0079	0.1153	0.1074	0.0690	0.0605	0.0779		-0.0013	0.0063
D’	-0.2791	0.4947	-0.6450	-0.2190	0.7789	0.5109	0.3159	0.3240	0.4312		-0.0133	1.0000
r^2	0.0090	0.0075	0.0848	0.0051	0.2382	0.2060	0.0868	0.0636	0.1017		0.0000	0.0095
P	0.1661	0.2066	<0.0001	0.2977	<0.0001	<0.0001	<0.0001	0.0002	<0.0001		0.9509	0.3196
KIR2DS5												
D	-0.0330	-0.0171	-0.0108	-0.0297	0.0314	0.1027	0.1284	0.1482	0.1190	-0.0065		-0.0039
D’	-0.7790	-0.4777	-0.1513	-0.7606	0.2517	0.5786	0.6961	0.9407	0.7609	-0.0593		-0.2877
r^2	0.0900	0.0283	0.0060	0.0788	0.0193	0.2051	0.3271	0.4160	0.2501	0.0009		0.0038
P	<0.0001	0.0138	0.2566	<0.0001	0.0421	<0.0001	<0.0001	<0.0001	<0.0001	0.6673		0.5284
KIR2DP1												
D	-0.0011	0.0131	0.0168	-0.0010	-0.0077	-0.0016	-0.0067	-0.0003	-0.0447	0.0067	-0.0037	
D’	-1.0000	0.7365	1.0000	-1.0000	-1.0000	-0.1440	-0.5885	-0.0360	-1.0000	1.0000	-0.2819	
r^2	0.0012	0.1906	0.1662	0.0011	0.0133	0.0006	0.0102	0.0000	0.0881	0.0107	0.0035	
P	0.6076	<0.0001	<0.0001	0.6227	0.0916	0.7304	0.1394	0.9398	<0.0001	0.1301	0.3889	
KIR3DP1												
D	-0.0003	-0.0002	0.0042	-0.0003	-0.0019	0.0019	-0.0028	0.0022	-0.0177	0.0017	0.0014	0.0046
D’	-1.0000	-1.0000	1.0000	-1.0000	-1.0000	1.0000	-1.0000	1.0000	-0.7908	1.0000	1.0000	1.0000
r^2	0.0003	0.0003	0.0410	0.0003	0.0033	0.0033	0.0073	0.0044	0.0260	0.0026	0.0020	0.2465
P	0.7988	0.8155	0.0031	0.8070	0.4022	0.3977	0.2124	0.3344	0.0182	0.4524	0.5079	<0.0001
	Macedonians (n = 214)											

[a] *D*, The classical linkage disequilibrium coefficient measuring deviation from random association between alleles at different loci; *D*’, the linkage disequilibrium coefficient *D* standardized by the maximum value it can take (D_{max}), given the allele frequencies; r^2, another way to standardise the simple measure of linkage disequilibrium [29]; *P*, statistical significance. &, not calculated for Macedonian Albanians.

frequencies (GF) were calculated using the formula GF=1-√(1-F), being aware of the limitation in its ability to detect *KIR* genes present at low frequency. For analysis of the molecular polymorphism of the locus studied, the Arlequin software version 3.0 [25] was used.

Linkage disequilibrium (LD) values for two locus associations were calculated using 2×2 tables [26]. Because LD is not independent of allele frequencies, normalized LD was calculated as described previously [27, 28]. Comparisons of different genotypes for two groups were tested by the χ2 test. Crude odds ratios (OR) were calculated within 95% CI. Previously published results for *KIR* gene polymorphisms in Macedonian population, [29] were used for comparison with *KIR* gene frequencies of the studied Albanian population.

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

Table 3: Pairs of *KIR* loci that displayed significant (p<0.05) LD in Macedonian Albanians (N = 104) and Macedonians (N=214).

	Macedonian Albanians (n = 104)											
	KIR3DL1	*KIR2DL1*	*KIR2DL3*	*KIR2DS4*	*KIR2DL2*	*KIR2DL5*	*KIR3DS1*	*KIR2DS1*	*KIR2DS2*	*KIR2DS3*	*KIR2DS5*	*KIR2DP1*
KIR3DL1		&	+	+	0	-	-	-	0	-	-	0
KIR2DL1	+		&	&	&	&	&	&	&	&	&	&
KIR2DL3	0	+		0	-	0	0	0	0	-	0	+
KIR2DS4	+	+	0		0	-	-	-	0	-	-	0
KIR2DL2	0	-	-	0		0	0	0	+	+	0	0
KIR2DL5	-	-	-	-	+		+	+	0	+	+	0
KIR3DS1	-	-	0	-	+	+		+	0	+	+	0
KIR2DS1	-	-	0	-	+	+	+		0	+	+	0
KIR2DS2	0	0	-	0	+	0	0	0		+	0	0
KIR2DS3	0	0	-	0	+	+	+	+	+		0	0
KIR2DS5	-	-	0	-	+	+	+	+	+	0		0
KIR2DP1	0	+	+	0	0	0	0	0	-	0	0	
KIR3DP1	0	0	+	0	0	0	0	0	-	0	0	+
	Macedonians (n = 214)											

0, no significant LD; +, significant positive LD; -, significant negative LD; &, LD not calculated.

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), *KIR3DL1* (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 ($D_{\max}$), 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 Allelefrequencies *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).

Haplo group	Genotype ID	*KIR 3DL1*	*KIR 2DL1*	*KIR 2DL3*	*KIR 2DS4*	*KIR 2DL2*	*KIR 2DL5*	*KIR 3DS1*	*KIR 2DS1*	*KIR 2DS2*	*KIR 2DS3*	*KIR 2DS5*	*KIR 2DL4*	*KIR 3DL2*	*KIR 3DL3*	*KIR 2DP1*	*KIR 3DP1*	Macedonian Albanians No (F)	Macedonians No (F)	Pearson's P
AA	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	20 (0.192)	46 (0.215)	0.640
AA	180	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	2	1	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	7 (0.067)	11 (0.051)	0.565
Bx	3	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	4 (0.039)	4 (0.019)	0.291
Bx	4	1	1	1	1	1	0	0	0	1	0	0	1	1	1	1	1	17 (0.164)	27 (0.126)	0.461
Bx	5	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	3 (0.029)	8 (0.037)	0.546
Bx	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2 (0.019)	8 (0.037)	0.349
Bx	7	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	4 (0.039)	6 (0.028)	0.672
Bx	8	1	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	2 (0.019)	2 (0.009)	0.458
Bx	9	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1 (0.010)	5 (0.023)	0.398
Bx	10	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1	1	2 (0.019)	1 (0.005)	0.208
Bx	11	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	6 (0.028)	0.026
Bx	13	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1 (0.010)	6 (0.028)	0.294
Bx	14	1	1	1	1	0	0	1	0	0	0	0	1	1	1	1	1	1 (0.010)	3 (0.014)	0.741
Bx	15	1	1	1	1	0	0	0	1	0	0	0	1	1	1	1	1	1 (0.010)	9 (0.042)	0.120
Bx	16	1	1	1	1	0	0	1	1	0	0	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	18	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1 (0.010)	2 (0.009)	1
Bx	19	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1 (0.010)	1 (0.005)	0.601
Bx	23	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	28	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	31	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	33	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	41	1	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	62	1	1	1	1	1	0	0	0	1	1	0	1	1	1	1	1	3 (0.029)	3 (0.014)	0.362
Bx	63	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1 (0.010)	2 (0.009)	1
Bx	68	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1 (0.010)	0	0.098
Bx	69	0	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1	0	2 (0.009)	0.306
Bx	70	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	3 (0.029)	2 (0.009)	0.190
Bx	71	1	1	0	1	1	1	0	0	1	1	0	1	1	1	1	1	2 (0.019)	4 (0.019)	1
Bx	72	1	0	0	1	1	0	0	0	1	0	0	1	1	1	0	1	0	1 (0.005)	0.367
Bx	73	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1 (0.010)	5 (0.023)	0.398
Bx	76	1	0	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0	2 (0.009)	0.306
Bx	79	1	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1 (0.010)	0	0.098
Bx	87	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1 (0.005)	0.470
Bx	88	0	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1 (0.010)	0	0.098
Bx	90	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	2 (0.019)	2 (0.009)	0.458
Bx	93	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1 (0.010)	0	0.098
Bx	113	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	118	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1 (0.010)	0	0.098
Bx	159	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	171	1	1	0	1	1	0	0	0	0	0	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	172	1	1	0	1	1	0	0	0	1	0	0	1	1	1	0	1	1 (0.010)	0	0.098
Bx	190	0	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	192	1	1	1	1	1	0	0	1	1	0	0	1	1	1	1	1	2 (0.019)	0	0.019
Bx	200	1	1	1	1	0	1	0	0	0	0	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	202	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	2 (0.019)	3 (0.014)	0.726
Bx	205	1	1	1	1	0	0	0	1	0	1	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	233	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1 (0.010)	5 (0.023)	0.398
Bx	260	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	0	2 (0.009)	0.306
Bx	268	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	294	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1 (0.010)	1 (0.005)	0.601
Bx	317	1	1	0	1	1	0	0	0	1	1	0	1	1	1	1	1	0	3 (0.014)	0.117
Bx	318	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	319	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1 (0.010)	2 (0.009)	1
Bx	331	1	1	1	0	0	1	1	1	0	1	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	336	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	359	1	0	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	363	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	370	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	3 (0.014)	0.117
Bx	371	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	372	1	1	1	1	0	0	1	1	0	1	0	1	1	1	1	1	2 (0.032)	1 (0.005)	0.208
Bx	373	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	0	2 (0.009)	0.306
Bx	374	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	375	0	1	1	0	0	0	0	1	0	0	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	376	0	0	0	1	1	0	0	1	1	1	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	377	0	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	0	3 (0.014)	0.117
Bx	378	1	1	0	1	1	0	1	0	1	0	0	1	1	1	0	0	0	1 (0.005)	0.367
Bx	379	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1 (0.005)	0.367
Bx	380	1	0	1	1	1	1	0	0	1	0	0	1	1	1	1	1	0	1 (0.005)	0.367
Bx	429	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1 (0.010)	0	0.098
Bx	new	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1 (0.010)	0	0.098
Bx	new	1	1	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	new	1	1	0	1	0	0	0	1	0	0	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	new	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1 (0.010)	0	0.098
Bx	new	1	1	0	0	1	0	0	0	1	1	0	1	1	1	1	1	1 (0.010)	0	0.098

KIR Genotype [1=Positive. 0=negative]

populated the south-western 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].

Table 5: AA and Bx *KIR* haplogroup frequencies in Macedonian Albanians (N = 104) and Macedonians (N = 214).

Haplogroup	Albanians		Macedonians		Pearson's p-value	Odds ratio	Wald 95% CI
	N	F	N	F			
AA	21	0.202	46	0.215	0.789	0.924	0.518-1.649
Bx	83	0.798	168	0.785			

N. number of individuals displaying AA or Bx *KIR* genotype; F. frequency of *KIR* genotype; CI. 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.

Acknowledgement

The authors thank Elena Cvetkovska (Institute of Immunobiology and Human Genetics, Faculty of Medicine, Ss. Cyril i Methodius University of Skopje, Skopje, Republic of Macedonia) for isolation of genomic DNA, and taking care of the Macedonian Human DNA Bank (http://www.hdna.mk).

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