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IL-1 Gene Cluster Polymorphisms in the Macedonian Population

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Abstract

The aim of this study was to genotype *IL-1* gene cluster polymorphisms in the Macedonian population. A group composed of 301 healthy unrelated individuals was selected. *IL-1* genotyping of the samples was performed by the PCR-SSP procedure followed by analysis of the *IL-1* data with the PyPop population genetic analysis software package. The frequency of *IL-1* alleles varies from 0.814 for *IL1A -889/C*, 0.729 for *IL-1B +3962/C*, 0.698 for *IL-1RN mspa 11100/T*, 0.671 for *IL-1B -511/C*, followed by 0.663 for *IL-1R psti 1970/C* indicating common "wild type" allele. We determined 27 different haplotypes from the total number of 32. The most frequent haplotypes for *IL-1* are *CCCCT* (0.191), *CTCCT* (0.127), *CCCTT* (0.098), *CCCCC* (0.085), *CTCTT* (0.082). All pairs of loci for *IL-1*, except for *IL-1R psti 1970*, are in linkage disequilibrium, with $p<0.05$. The closest genetic distance is observed between the studied Macedonian population and the population from ItalyTorino, while the most genetically distant populations were TaiwanTsou, and TaiwanYami. It is concluded that *IL-1* cluster alleles, genotypes, and haplotypes in Macedonian population show a good concordance with Hardy Weinberg equilibrium and can be used for anthropological comparisons, as well as for association studies with different diseases.

Introduction

Until recently, the *IL-1* ligand family consisted of four members of: *IL-1A, IL-1B, IL-1RA*, and *IL-18*. Six additional members of this family have been described since. The entire new gene map of the region of chromosome 2 between the *IL-1B* and *IL-1RN* loci was proposed (1), suggesting that each of the new *IL-1* family members arose from a common ancestral gene that later became duplicated (2). The novel *Il-1* family members have been described by several groups using their own nomenclature, thus resulting in a number of different names for the same molecule (3, 4).

The prototypic members of the *IL-1* family gene cluster are the *IL-1A* (MIM 147760), *IL-1B* (MIM 147720), and *IL-1RN genes. IL-1A* and *-1B* encode proinflammatory cytokines involved in host defense against infection. The *IL-1* receptor antagonist, encoded by the gene *IL-1RN*, is an anti-inflammatory nonsignalling molecule that competes for receptor binding with *IL-1A* and *IL-1B* (5, 6). Six genes with structural homology to *IL-1A/B* or *IL-1RN* lie between *IL-1A* and *IL-1RN*. They are named *IL-1F5* (MIM 605507), *IL-1F6* (MIM 605509), *IL-1F7* (MIM 605510), *IL-1F8* (MIM 605508), *IL-1F9* (MIM 605542), and *IL-1F10*; *IL-1A, IL-1B,* and *IL-1RN* have been renamed *IL-1F1, IL-1F2,* and *IL-1F3,* respectively (7, 8). The genes are ordered, from centromere to telomere, as *IL-1A, IL-*

1B, IL-1F7, IL-1F9, IL-1F6, IL-1F8, IL-1F5, IL-1F10, IL-1RN, in a cluster covering a region of ~360-kb region (9).

The protein encoded by *IL1R1* gene is a cytokine receptor that belongs to the interleukin 1 receptor family. This protein is a common receptor for interleukin alpha (*IL1A*), interleukin beta (*IL1B*), and interleukin 1 receptor, type I (*IL1R1/IL1RN*) and is an important mediator involved in many cytokine induced immune and inflammatory responses. It has been shown by radiation hybrid mapping that *IL1R1, IL1R2* (MIM 147811), *IL1RL2* (MIM 604512), *IL1RL1* (MIM 601203), and *IL18R1* (MIM 604494) map to 2q12 and are transcribed in the same direction with *IL1R2* and form a cytokine receptor gene cluster (10).

Cytokines are potent immunomodulating molecules that mediate the inflammation and immune response, and influence cellular activation, differentiation, and function. There are many reports showing that a number of cytokine genes are polymorphic and that polymorphisms in the gene regulatory regions correlate with the level of cytokine secretion (11, 12). As these polymorphisms are independently segregated, one individual may have a cytokine expression pattern quite different from each other (13).

Fourteen cytokine genes (22 SNP alleles) were identified as the candidates for the cytokine polymorphism component (CPC) at the 13th International Histocompatibility Workshop and Congress (IHWC) (14). Five cytokine polymorphisms, related to the *IL-1* cluster gene on chromosome 2, were included in the cytokine polymorphism component i.e; *IL-1A -889, IL-1B -511, IL-1B +3962, IL-1R psti 1970*, and *IL-1RN mspa 11100*.

The aim of this study was to obtain specific data of *IL-1* cluster gene polymorphisms in Macedonian population that can be used as useful anthropological markers and serve as a basis for disease association studies.

Methods

Population

The studied population consists of 301 healthy unrelated individuals, aged between 20-35 years. All individuals are of Macedonian origin and nationality and residents of different geographical regions of the Republic of Macedonia. The spoken language is Macedonian. 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. Individuals with only one Macedonian parent were excluded from the study .The participants were informed that they can withdraw or destroy their deposited DNA samples in any time. Blood samples were collected, DNA was isolated from peripheral blood leukocytes by the phenol-chlorophorm extraction method (15, 16), samples were anonymized for privacy, and stored in the Macedonian Human DNA Bank (hDNAMKD) (17).

Typing Methods

Cytokine genotyping for the anthropology samples was performed by PCR-SSP (Heidelberg kit/ CST-PCR-SSP Tray, obtained from the University clinic of Heidelberg, University of Heidelberg, Heidelberg, Germany). Briefly, PCR-SSP typing by the Heidelberg kit represents 48 PCR primer mixes aliquotted in 96 well PCR trays (two typings per tray). Master mix, which was supplied along with the reagents and consisted of $MgCL_2$, buffer, dNTP's, and glycerol was mixed with 1.2 – 3.0 µg DNA and 20 U Taq polymerase and dispensed in the 48 wells. Agarose gel electrophoresis on a 2% agarose of an aliquot of the obtained products, revealed a positive or a negative specific amplification for each well (18). Subsequently, the results were entered in the Cytokine-SCORE software and analyzed automatically (19). A manual interpretation was also performed according to the interpretation scheme provided along with the kit.

Statistical Methods

The population genetics analysis package, PyPop (20-22), was used for analysis of the cytokine data for this report. Allele frequencies and expected Hardy Weinberg proportions (HWP) for each SNP were determined (23). The exact test for genotype frequency deviation from HWP was calculated using the Arlequin implementation accessed via PyPop (24). Those SNPs that did not fit HWP were evaluated to determine whether there was an excess of homozygotes or heterozygotes, or if any particular genotypes were significantly different from expected frequencies by the chi square test. The Ewens-Watterson homozygozity test of neutrality (25) with Slatkins' p-values (26, 27) was used to indicate any deviations from the hypothesis of neutral selection for each locus. The statistic for the Ewens-Watterson homozygozity test of neutrality is the F statistic. If the Normal Deviate of Homozygozity (F_{nd}) is significantly less than 0, it indicates balancing selection is operating on the alleles at that locus;

significant $F_{nd} > 0$ indicates directional selection (28), provided the assumptions of the model are met. P-values less than 0.025 or greater than 0.975 are considered to be significant for this 2-tailed test at the 0.05 level. For the all *IL-1* SNPs, test of neutrality showed negative value for F_{nd} statistic (Ewens-Watterson test of neutrality) which indicates balancing selection operating on the *IL-1* alleles, but without significant p of F statistics.

Linkage disequilibrium (LD) was calculated, where *D'* weights the contribution to LD of specific allele pairs by the product of their allele frequencies (29); W_n is a re-expression of the chi-square statistic for deviations between observed and expected haplotype frequencies; *S* is defined as twice the difference between log-likelihood of obtaining the observed data given the inferred haplotype frequencies [*ln(L_1)*], and the likelihood of the data under the null hypothesis of linkage equilibrium [*ln(L_0)*]); *p*-value is the fraction of permutations that results in values of *S* greater or equal to that observed.

The DISPAN software was used for computation of the genetic distances between the studied population and other populations for which a complete data for *IL-1* gene cluster was available (30). Phylogenetic and molecular evolutionary analyses were conducted using MEGA software version 3.1 (31).

Results

IL-1 Alleles

Frequencies of polymorphic *IL-1* alleles, test of neutrality with Fnd statistic [Ewens-Watterson test of neutrality (EWN)], and Slatkin's Exact P Value (SEPV) with p of F statistics in the studied population are shown on the Table 1. The frequency of *IL-1* alleles varies from 0.814 for *IL-1A -889/C*, 0.729 for *IL-1B +3962/C*, 0.698 for *IL-1RN mspa 11100/T*, 0.671 for *IL-1B -511/C*, followed by 0.663 for *IL-1R pstI 1970/C* indicating common "wild type" allele in those cytokines (Table 1).

IL-1 genotypes

Observed versus expected cytokine genotype frequencies for each *IL-1* SNP, Hardy Weinberg proportion (HWP), and Guo and Thompson Hardy Weinberg Output (GTHWO) in Macedonian population are given on the Table 2. Several observed frequencies of *IL-1* genotypes were significantly different from the expectations: *IL-1A -889/T:T* (p=0.015), *IL-1B +3962/C:T* (0.011), and *IL-1B +3962/T:T* (p=0.003). The rest of *IL-1* genotypes showed a good concordance with HWP expectations. Two *IL-1* SNPs (*IL-1A -889*, and *IL-1B +3962*) were not in HWP (p<0.003, and <0.001, respectively), and Guo and Thompson Hardy Weinberg Output (GTHWO) was significant (p<0.006, and <0.001, respectively) (Table 2).

Table 1: Frequencies of polymorphic *IL-1* cluster alleles, test of neutrality with Fnd statistic (Ewens-Watterson test of neutrality (EWN)), and Slatkin's Exact P. Value (SEPV) with p of F statistics in Macedonian population.

Il-1 Polymorphism	Alleles			Test of Neutrality	
				EWN	SEPV
	Allele	Number	Frequency	F_{nd}	*P* of F
IL-1A -889	*C*	482	0.814	-0.967	0.214
	T	110	0.186		
IL-1B -511	*C*	404	0.671	-1.809	0.103
	T	198	0.329		
IL-1B +3962	*C*	439	0.729	-1.527	0.141
	T	163	0.271		
IL-1R psti 1970	*C*	399	0.663	-1.843	0.096
	T	203	0.337		
IL-1RN mspa 11100	*T*	420	0.698	-1.691	0.119
	C	182	0.302		

Statistically significant $F_{nd} < 0$ indicates balancing selection; significant $F_{nd} > 0$ indicates directional selection.

IL-1 Linkage Disequilibrium (LD)

Linkage disequilibrium (LD) measures for each pair of loci for *IL-1* are presented on Table 3. One can see from the Table 3 that all pairs of loci for *IL-1*, except for *IL-1R psti 1970*, are in linkage disequilibrium, with p<0.05.

IL-1 Haplotypes

Haplotype frequency estimated for *IL-1* loci and number of copies is shown on the Table 4. Haplotype frequencies were estimated from unphased data using the expectation-maximization (EM) algorithm (21, 28) reported by PyPop. *IL-1B* had 2 SNPs individually typed by the Heidelberg PCR-SSP kit, but the kit was not designed to detect haplotypes for these SNPs. We estimated 27 different haplotypes from the total number of 32. The most frequent haplotypes (more than 5%) for *IL-1* are *CCCCT* (0.191), *CTCCT* (0.127), *CCCTT* (0.098), *CCCCC* (0.085), *CTCTT* (0.082). The less frequent haplotype was *CTTCC* (0.002). Five possible *IL-1* haplotypes are missing in Macedonian population (Table 4).

Table 2: Observed vs. expected *IL-1* cluster genotypes for each SNP, Hardy Weinberg proportions (HWP), and Guo and Thompson Hardy Weinberg Output (GTHWO) in Macedonian population.

IL-1 Polymorphism	Genotype	Observed	Observed frequency (%)	Expected	*P*-value	HWP *P*-value	GTHWO *P*-value
IL-1A -889	*C:C*	204	68.9	196.2	0.579	<0.003*	<0.006*
	C:T	74	25.0	89.6	0.100		
	T:T	18	6.1	10.2	0.015*		
IL-1B -511	*C:C*	143	47.5	135.6	0.523	0.052	0.052
	C:T	118	39.2	132.9	0.197		
	T:T	40	13.3	32.6	0.192		
IL-1B +3962	*C:C*	174	57.8	160.0	0.271	<0.001*	<0.001*
	C:T	91	30.2	118.9	0.011*		
	T:T	36	12.0	22.1	0.003*		
IL-1R psti1970	*C:C*	133	44.2	132.2	0.946	0.840	0.898
	C:T	133	44.2	134.5	0.894		
	T:T	35	11.6	34.2	0.895		
IL-1RN mspa11100	*C:C*	30	10.0	27.5	0.635	0.497	0.499
	C:T	122	40.5	127.0	0.659		
	T:T	149	49.5	146.5	0.837		

*, Statistically significant.

IL-1 Genetic Distances

We used data from 26 populations published on the WEB (32), selected on the basis of presence of *IL-1* gene cluster frequencies. The names of the populations are given as they are deposited. For most of the populations, the heterozygosity was around 0.5, populations from SouthKoreaSeoul, Taiwan, and TurkeyBursa had heterozygosity around 0.4, and population from TaiwanAtayal had the smallest heterozygosity around 0.37. Standard genetic distance (SGD) between Macedonian population (present study) and different populations are given x 10^2 (Table 5).

Diversity of investigated populations for *IL-1* gene cluster is given for all loci, and locus by locus on the Table 6. Measure of population differentiation (G_{ST}) for all loci was 0.079, for the locus *IL-1B +3962* was the biggest (0.143), and the smallest was for locus *IL-1RN mspa11100* (0.046). Total genetic diversity in the

Table 3: Linkage disequilibrium (LD) measures for each pair of loci of *IL-1* cluster genes.

		IL-1A -889	*IL-1B -511*	*IL-1B +3962*	*IL-1R psti 1970*	*IL-1RN mspa 11100*
IL-1A -889						
IL-1B -511	D'	0.45745				
	Wn	0.15257				
	S	8.56				
	p	0.002*				
IL-1B +3962	D'	0.58741	0.41033			
	Wn	0.46110	0.17504			
	S	72.25	12.99			
	p	<0.001*	0.001*			
IL-1R psti 1970	D'	0.18242	0.08051	0.15419		
	Wn	0.06225	0.07902	0.06701		
	S	1.44	2.21	1.77		
	p	0.225	0.150	0.189		
IL-1RN mspa 11100	D'	0.19685	0.32096	0.18452	0.09878	
	Wn	0.14171	0.14791	0.1708	0.09116	
	S	7.23	8.81	10.90	2.36	
	p	0.009*	0.004*	0.001*	0.124	

D' weights the contribution to LD of specific allele pairs by the product of their allele frequencies (30); W_n is a re-expression of the chi-square statistic for deviations between observed and expected haplotype frequencies; *S* is defined as twice the difference between log-likelihood of obtaining the observed data given the inferred haplotype frequencies [*ln(L_1)*], and the likelihood of the data under the null hypothesis of linkage equilibrium [*ln(L_0)*]); *p*-value is the fraction of permutations that results in values of *S* greater or equal to that observed. A *p*-value < 0.05 is indicative of overall significant LD. *, statistically significant.

Table 4: Haplotype frequency estimated for *IL-1* cluster loci: *IL-1A -889: IL-1B -511: IL-1B +3962: IL-1R psti1079: IL-1RN mspa11100*.

Haplotype		Frequency	Number of Copies
1.	CCCCT	0.191	113.3
2.	CTCCT	0.127	75.0
3.	CCCTT	0.098	58.3
4.	CCCCC	0.085	50.1
5.	CTCTT	0.082	48.5
6.	CCTCT	0.048	28.3
7.	TCTCT	0.047	27.7
8.	CCCTC	0.038	22.5
9.	TCTCC	0.037	22.0
10.	CTCCC	0.033	19.4
11.	TCCCT	0.030	17.9
12.	TCTTC	0.027	15.8
13.	CCTCC	0.024	14.3
14.	CTCTC	0.020	12.1
15.	CTTCT	0.020	11.7
16.	CCTTC	0.017	10.1
17.	CCTTT	0.015	9.1
18.	TCCTC	0.011	6.5
19.	CTTTT	0.010	5.8
20.	TTTCT	0.008	4.8
21.	*TTCTT*	0.008	4.6
22.	*TTCCT*	0.007	3.9
23.	*TTTCC*	0.004	2.6
24.	*CTTTC*	0.004	2.5
25.	*TTTTC*	0.004	2.1
26.	*TCTTT*	0.003	2.1
27.	*CTTCC*	0.002	1.1

Haplotype frequencies were estimated from unphased data using the expectation-maximization (EM) algorithm (22, 29) reported by PyPop. *IL-1B* had 2 SNPs individually typed by the Heidelberg PCR-SSP kit, but the kit was not designed to detect haplotypes for these SNPs. Number of individuals: 301 (before-filtering); Number of individuals: 296 (after-filtering); Unique phenotypes: 95; Unique genotypes: 244; Number of haplotypes: 32; Loglikelihood under linkage equilibrium [ln(L_0)]: -1379.169; Loglikelihood obtained via the EM algorithm [ln(L_1)]: -1315.539; Number of iterations before convergence: 56.

pooled populations (H_T) for all loci was 0.561, the biggest was for locus *IL-1B -511* (0.630), and the smallest was for the locus *IL-1B +3962* (0.478). Mean diversity within each population (H_S) for all loci was 0.516, the biggest was for the locus *IL-1B -511* (0.598), and the smallest was for the locus *IL-1B +3962* (0.409) (Table 6).

The Neighbor-Joining phylogenetic tree was constructed on the basis of comparison of allele frequencies for *IL-1* gene cluster in different populations. The closest genetic distance is observed between the studied Macedonian population and the ItalyTorino (SGD = 1.44), while the most genetically distant populations were TaiwanTsou (SGD=17.43), and TaiwanYami (SGD=15.10) (Table 5). Populations from Taiwan (six different populations), from TurkeyBursa and from SouthKoreaSeoul are in the same cluster on the bottom of the tree. Populations from IndiaNorth and GreeceMacedoniaandTraceRegions are in the same cluster above the bottom cluster. Above is the cluster with IranSouteastSistani and BrasilSaoPaolo. European populations are on the opposite side of the genetic tree (CzechRepublicMoravia, Greece, PortugalCentre, IrelandSouth and Germany). Populations from ItalyTorino and from Macedonia are in the same cluster, between the European populations and the cluster of populations from Iran (five populations), and from IndiaDelhi. The population from Cyprus is isolated and is situated between the cluster with Iranian populations, and the cluster of IranSouteastSistani and BrasilSaoPaolo (Fig. 1).

Table 5: Investigated populations, number of individuals, heterozygousity, and standard genetic distances (SGD) between Macedonian population (present study) and different populations (www.allelefrequencies.net) using *IL-1* gene cluster frequencies (32).

Population	Number of individuals	Heterozygosity ± SE	SGD (x 10^2)
1. BrazilSaoPaolo	99	0.538 ± 0.043	4.99
2. Cyprus	100	0.549 ± 0.020	3.38
3. CzechRepublicMoravia	67	0.554 ± 0.011	5.82
4. Germany	200	0.571 ± 0.008	2.50
5. Greece	120	0.568 ± 0.017	5.64
6. GreeceMacedoniaand-ThraceRegions	27	0.524 ± 0.026	5.71
7. IndiaDelhi	34	0.559 ± 0.034	6.19
8. IndiaNorth	130	0.512 ± 0.052	9.53
9. Iran	40	0.559 ± 0.021	3.91
10. IranSoutheastBaloch	96	0.550 ± 0.038	7.01
11. IranSoutheastSistani	98	0.547 ± 0.053	5.39
12. IranTehran	140	0.568 ± 0.015	4.41
13. IranTehranFarsi	40	0.577 ± 0.009	3.96
14. IranYazd	121	0.542 ± 0.016	5.45
15. IrelandSouth	200	0.564 ± 0.016	3.15
16. ItalyTorino	140	0.558 ± 0.012	1.44
17. Macedonia	301	0.568 ± 0.027	-
18. PortugalCentre	174	0.558 ± 0.016	3.06
19. SouthKoreaSeoul	57	0.418 ± 0.093	13.27
20. TaiwanAmi	50	0.499 ± 0.073	5.76
21. TaiwanAtayal	50	0.373 ± 0.119	12.31
22. TaiwanHakka	45	0.447 ± 0.112	13.00
23. TaiwanMinnan	50	0.468 ± 0.109	12.65
24. TaiwanTsou	50	0.421 ± 0.115	17.43
25. TaiwanYami	40	0.434 ± 0.111	15.10
26. TurkeyBursa	60	0.495 ± 0.047	12.03

Table 6: Diversity of investigated populations for *IL-1* cluster genes locus by locus.

Locus	G_{ST}*	H_T	H_S
IL-1A -889	0.099	0.520	0.469
IL-1B -511	0.051	0.630	0.598
IL-1B +3962	0.143	0.478	0.409
IL-1R psti1970	0.076	0.571	0.527
IL-1RN mspa11100	0.046	0.604	0.577
All loci	0.079	0.561	0.516

*, G_{ST}, measure of population differentiation; H_T, total genetic diversity in the pooled populations; H_S, mean diversity within each population.

Discussion

This report summarizes *IL-1* gene cluster polymorphisms and variations that exist in Macedonian population.

With the test of neutrality we found negative F_{nd} for *IL-1* cluster genes, but without significant p of F statistics, which indicates balancing selection operating on the alleles at that cluster. We found also that most of *IL-1* genes showed a good concordance with HWP expectations. Two *IL-1* genes (*IL-1A -889*, and *IL-1B +3962*) were not in HWP, and Guo and Thompson Hardy Weinberg Output was significant and could be as a result of existence of selective pressures, or because of the small frequencies in the investigated groups.

Inter-population discrepancies in allele frequencies particularly between Caucasian and non-Caucasian sample cohorts are often large (33). Evaluation of the allele frequencies of the Dutch, Italian and Czech populations showed that five SNPs were significantly different between the Dutch and the Italians, while these SNPs did not vary between the Dutch and the Czechs (34). Significant differences in allelic frequencies among ethnic groups were reported (35). The *IL-1RN* allele 2 was very rare in Koreans (frequency, 0.060). In addition, it was also found a significant

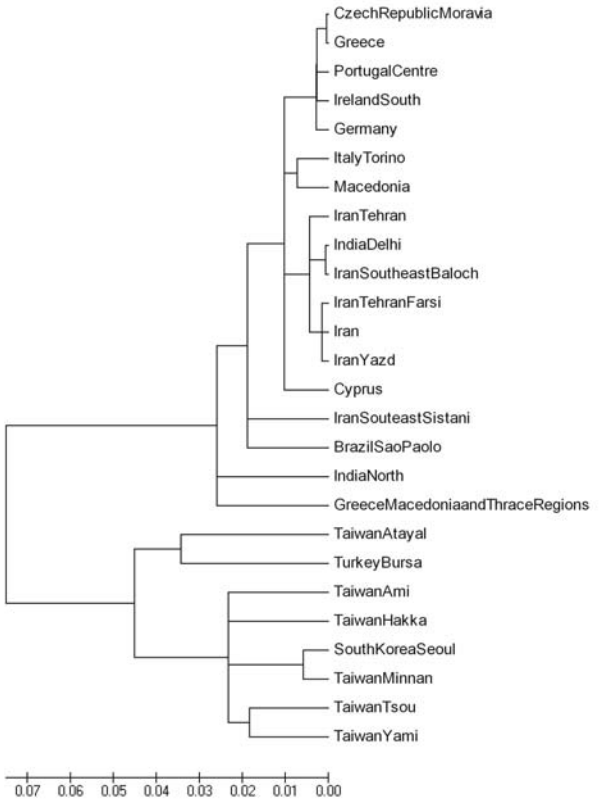

Figure 1: Neighbor-Joining phylogenetic tree, constructed on the basis of standard genetic distances of IL-1 *gene cluster, shows the relations between Macedonian and other populations (www.allelefrequencies.net) (Middleton et al., 2003).*

difference for the *IL-1A* (-889) and *IL-1B* (+3953) polymorphisms in Koreans compared with Caucasians (36). The frequency of *IL-1RN* 2-repeat allele was significantly lower in Taiwanese than in Caucasians. In contrast, the frequencies of the pro-inflammatory *IL-1B –511T* allele and *+3954C* allele were significantly higher among Taiwanese compared with Caucasians (37).

In some countries, where large ethnic populations exist in dense clusters, these observations should be taken into account when designing a high impact association study. It is particularly important to achieve accurate matched population profiling in both, case control and cohorts, as small differences can provide positive associations brought about by ethnic mismatch rather than disease susceptibility.

We found significant association between all pair of loci (LD had p less than 0.05), except for *IL-1R psti 1970* which was not significantly associated with any of the *IL-1* cluster loci. Haplotype frequencies estimated for *IL-1* cluster loci have shown 27 haplotypes, from 32 possible, meaning that 5 haplotypes of *IL-1* cluster genes are not present in Macedonian population. The most frequent haplotypes were *CCCCT* (0.191), and *CTCCT* (0.127); and most rare haplotypes were *TCTTT* (0.003), and *CTTCC* (0.002).

Published results indicated that after the exposure to LPS, whole blood leukocytes from subjects with the homozygous *IL-1B* haplotype *-1470G, -511C*, and *-31T (GCT)* produced more IL-1b in vitro than those from subjects with haplotype *-1470C, -511T,* and *-31C (CTC)* and that the transcriptional activity of the haplotype *GCT* was also higher than that of the haplotype *CTC* (38). It is suggested that the haplotypes of the *IL-1B* promoter influence the expression and transcriptional activity of the *IL-1B* gene and that the upregulation of *IL-1B* gene expression after LPS exposure in subjects with haplotype *GCT* may be due to an increased transcriptional activity of that haplotype (38). Since the effect of *IL-1B* polymorphisms on IL-1b production is still controversial, two polymorphisms were selected to test their cis-acting effect on IL-1b mRNA expression by means of the allele-specific transcript quantification and the haplotype analysis. As for the *C-31T* polymorphism, it was found that expression of the *-31T* allele was 2.2 times greater then that of the *-31C* allele. This higher transcription efficiency may correspond to the fact that *C-31T* is located in a TATA box. The other polymorphism, *C+3954T*, did not alter the levels of transcription (39).

Under treatment with 10^{-4}M prednisolone, the levels of IL-1b protein production stimulated by LPS in PBMC extracted from the subjects with the *IL-1*B TT-31, TC-31, and *CC-31* genotypes were suppressed to 6.0±3.4%, 31.4±57.0%, and 87.7±84.8%, respectively, of the level in prednisolone-untreated control cells (*TT-31 vs. CC-31*, $p<0.05$). Glucocorticoid-based anti-inflammatory therapy might be less effective in patients with the *IL-1*B TC-31 and *CC-31* genotypes than those with the *TT-31* genotype (40).

In general, an understanding of which markers are in strong linkage disequilibrium allows for the more rational design of genetic studies. In the *IL-1* system in particular, where alleles of different *IL-1* genes may act in concert to determine an overall inflammatory phenotype, knowledge of the existing disequilibria is vital to our understanding of which allele combinations are important in disease.

We report here, for the first time, genetic distances of *IL*-1 gene cluster polymorphism for 26 populations, deposited on the allelefrequencies.net (32), and they group in several clusters: Taiwan

populations on the bottom of the tree, and European populations on the top of the tree. Macedonian population is situated in the same cluster with the population of ItalyTorino, between the cluster of European populations and cluster with Iranian populations. One could speculate that diferent selective pressures in the past might have contributed to the population positions in the clusters.

In summary, the results of *IL-1* cluster alleles, genotypes, estimated haplotypes, and linkage disequilibrium in Macedonian population are similar with the published data for cytokine polymorphism, and can be used for anthropological comparisons, as well for association studies with different diseases.

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