Original Article

Distribution of cytokine gene single nucleotide polymorphisms among a multi-ethnic Iranian population

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Abstract

Background: Cytokine gene single nucleotide polymorphisms (SNPs) are widely used to study susceptibility to complex diseases and as a tool for anthropological studies.

Materials and Methods: To investigate cytokine SNPs in an Iranian multi-ethnic population, we have investigated 10 interleukin (IL) SNPs (IL-1β (C-511T, T-31C), IL-2 (G-384T), IL-4 (C-590T), IL-6 (G-174C), IL-8 (T-251A), IL-10 (G-1082A, C-819T, C-592A) and tumor necrosis factor-alpha (TNF-α) (G-308A) in 415 Iranian subjects comprising of 6 different ethnicities. Allelic and genotypic frequencies as well as Hardy-Weinberg equilibrium (HWE) were calculated by PyPop software. Population genetic indices including observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{IS}), the effective number of alleles (N_e) and polymorphism information content (PIC) were derived using Popgene 32 software. Multidimensional scaling (MDS) was constructed using Reynold's genetic distance obtained from the frequencies of cytokine gene polymorphism.

Results: Genotypic distributions were consistent with the HWE assumptions, except for 3 loci (IL-4-590, IL-8-251 and IL-10-819) in Fars and 4 loci (IL-4-590, IL-6-174, IL-10-1082 and TNF-α-308) in Turks. Pairwise assessment of allelic frequencies, detected differences at the IL-4-590 locus in Gilakis versus Kurds ($P = 0.028$) and Lurs ($P = 0.022$). Mazanis and Gilakis displayed the highest ($H_o = 0.50 \pm 0.24$) and lowest ($H_o = 0.34 \pm 0.16$) mean observed heterozygosity, respectively.

Conclusions: MDS analysis of our study population, in comparison with others, revealed that Iranian ethnicities except Kurds and Mazanis were tightly located within a single cluster with closest genetic affinity to Europeans.

Key Words: Allelic frequency, Arlequin, genetic diversity, polymorphism information content, single nucleotide polymorphisms

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INTRODUCTION

Iran (Persia) is a multi-ethnic country located in the Middle East. It is bordered on the north by the Caspian Sea and south by the Persian Gulf and the Gulf of Oman. Its population is comprised of genetically heterogeneous groups, but the exact ethnic composition of Iran remains loosely defined. Accordingly,

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Persians (Fars) constitute 61% of the population of Iran, followed by Turks (Azeri, 16%), Kurds (10%), Lurs (6%), Arabs (2%), Balochs (2%), Turkmens and Turkic tribes (2%), and other ethnicities (1%). The official language of Iran is Persian (Farsi), which is spoken by 53% of the population. Other languages include Turkish (18%), Kurdish (10%), Gilaki/Mazani (7%), Luri (6%), Balochi (2%), Arabic (2%) and others (2%).[1,2] Characterization of genetic profiles and analysis of differences between ethnic groups should provide a better understanding of their disparities and genetic susceptibility to disease. Such analyses are usually conducted using histocompatibility leukocyte antigens (HLAs),[3] cytokine gene polymorphisms[4] and other genetic markers such as microsatellites.[4-7] Cytokines are key immune-modulatory molecules which regulate the activities of multiple target cells via binding to specific receptors and are involved in the pathogenesis of numerous diseases.[2] A number of functional polymorphisms within the regulatory regions of cytokine genes affect gene transcription, causing variations in their level of production.[8,9] The role of cytokine gene polymorphisms in screening for susceptibility to inflammatory diseases, transplant rejection, autoimmunity and various cancers has been vastly studied.[10-13] Cytokine gene polymorphisms comprise of single nucleotide polymorphisms (SNPs), microsatellite polymorphisms, gene insertions and deletions.[7,10,14] Their distribution varies significantly among different ethnic groups, which in turn may contribute to the observed differences in ethnicity-dependent disease prevalence.[6,15-17] The purpose of the current study was to investigate the genomic variation of a range of cytokine SNPs (IL-1β-511, IL-1β-31, IL-2-384, IL-4-590, IL-6-174, IL-8-251, IL-10-592, IL-10-819 and IL-10-1082). The studied cytokines were selected from the categories of pro- and anti-inflammatory cytokines, which are most associated with the development of various diseases, particularly cancers. In addition to the calculation of genetic diversity, similarities and differences among all tested groups and other worldwide populations was evaluated using MDS analysis.

MATERIALS AND METHODS

Study population

In order to investigate cytokine functional SNP distribution, in the multi-ethnic population of Iran, we have assessed 415 unrelated healthy individuals from six major Iranian ethnic groups [Figure 1]. The study population comprised of 198 Fars, 139 Turk, 32 Gilaki, 23 Lur, 14 Kurd and 9 Mazani ethnic subjects. Geographical origins and ethnicities were determined by personal interview. Subjects were included whose parents were of the same ethnic groups. This study, including data and sample collection, was carried out following provision of written informed consents by the participants, according to protocols approved by the National Committee on Ethical Issues in Medical Research, Ministry of Health and Medical Education of Iran; Ref No. 315.

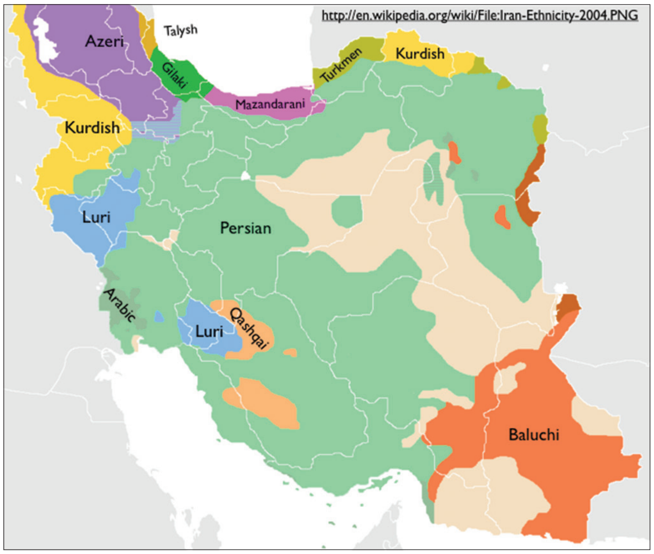

Figure 1: Geographic distribution of Iranian ethnic groups. Colors indicate different ethnicities

Cytokine genotyping

Venous blood (5 ml) was obtained from each subject for genetic studies. Genomic DNA was extracted from white blood cells using salting out extraction method.[18] The quality and quantity of DNA were determined by spectrophotometric measurement of absorbance at 260/280 nm, and the extracted DNAs were stored at –20°C for further processing.

Various PCR based methods including CTPP (confronting two-pair primers) and RFLP (restriction fragment length polymorphism) were used to identify SNPs in the following cytokine genes: IL-1β (C-511T, T-31C), IL-2 (G-384T), IL-4 (C-590T), IL-6 (G-174C), IL-8 (T-251A), IL-10 (G-1082A, C-819T, C-592A) and TNF-α (G-308A) [Table 1].

Briefly, the genomic DNA was amplified with specific primers [Table 1]. Each 20 μl of PCR reaction contained 50 ng DNA, 25 pM of each primers, 0.2 mM of each dNTPs, 1X PCR buffer, 1.5 mM $MgCl_2$ and 1 U Taq Polymerase (CinnaGen, Iran). PCR products were digested 3–16 h with suitable restriction enzymes at 37°C. PCR products and the resulting digested fragments were visualized by agarose gel electrophoresis. For each studied SNP, three randomly selected wild type and mutant samples were sequenced. Blinded cross-checking of PCRs, and random sequence analysis of PCR products validated the genotyping results.

Table 1: Primer sequences and methods used for detection of cytokine gene polymorphisms

Gene	Method	Primers and PCR conditions	Restriction Enzymes	Products	Ref.
IL-1β C-511T (rs16944)	PCR-RFLP	F=5'- TGGCATTGATCTGGTTCATC -3' R=5'- GTTTAGGAATCTTCCCACTT -3' 94°C (5 min)+[94°C (1 min)+57°C (1 min)+72°C (1 min)]×30+72°C (5 min)	*Ava*I	190+115 bp=CC 305 bp=TT 305+190+115 bp=CT	[43]
IL-2 G-384T (rs36215458)	PCR-RFLP	F=5'- TATTCACATGTTCAGTGTAGTTCT -3' R=5'- CATTGTGGCAGGAGTTGAGGT -3' 94°C (4 min)+[94°C (1 min)+60.5°C (1 min)+72°C (1 min)]×30+72°C (3 min)	*Bfa*I	414 bp=TT 414+389 bp=GT 389 bp=GG	[12]
IL-4 C-590T (rs2243250)	PCR-RFLP	F=5'- TAAACTTGGGAGAACATGGT -3' R=5'- TGGGGAAAGATAGAGTAATA -3' 94°C (3 min)+[94°C (30 sec)+53.5°C (30 sec)+72°C (30 sec)]×35+72°C (3 min)	*Ava*II	195 bp=CC 175+20 bp=TT 195+175+20 bp=CT	[44]
IL-6 G-174C (rs1800795)	PCR-RFLP	F=5'- TGCCAAAGTGCTGAGTCACT -3' R=5'- ATCCCACATTTGATAAATC -3' 94°C (5 min)+[94°C (1 min)+59°C (1 min)+72°C (1 min)]×30+72°C (3 min)	*Nla*III	102+124 bp=CC 226 bp=GG 102+124+226 bp=GC	*
TNF-α G-308A (rs1800629)	PCR-RFLP	F=5'- GAGGCAATAGGTTTTGAGGGCCAT -3' R=5'- GGGACACACAAGCATCAAG -3' 94°C (5 min)+[94°C (1 min)+ 59°C (1 min)+72°C (1 min)]×30+72°C (3 min)	*Nco*I	147 bp=AA 124+147 bp=GA 124 bp=GG	[45]
IL-1β C-31T (rs1143627)	CTPP	F1=5'- AATGTGGACATCAACTGCA -3' F2=5'- CTACTAAGGCTTCTTTGGGAA -3' R1=5'- CTCCCTCGCTGTTTTTATA -3' R2=5'- TCAGCTGTTAGATAAGCAG -3' 94°C (5 min)+[94°C (1 min)+54°C (1 min)+72°C (1 min)]×30+72°C (5 min)	-	574+345 bp=CC 574+266 bp=TT 574+345+266 bp=CT	[43]
IL-8 T-251A (rs4073)	CTPP	F1=5'- CATGATAGCATCTGTAATTAACTG -3' F2=5'- GTTATCTAGAAATAAAAAAGCATACAA -3' R1=5'- CACAATTTGGTGAATTATCAAA -3' R2=5'- CTCATCTTTTCATTATGTCAGAG -3' 94°C (5 min)+[94°C (2 min)+56°C (1 min)+72°C (1 min)]×35+72°C (5 min)	-	348+168 bp=TT 348+228 bp=AA 348+228+168 bp=TA	[46]
IL-10 G-1082A (rs1800896)	CTPP	F1=5'-TCCAGATATCTGAAGAAGTCCTG -3' F2=5'-CTACTAAGGCTTCTTTGGGAA-3' R1=5'-TTACCTATCCCTACTTCCCCC-3' R2=5'-CAGTGCCAACTGAGAATTTGG-3' 94°C (5 min)+[94°C (2 min)+56.1°C (1 min)+72°C (1 min)]×35+72°C (10 min)	-	414+259 bp=AA 414+199 bp=GG 414+259+199=AG	[47-50]
IL-10 C-819T (rs1800871)	CTPP	F1=5'- TCCAGATATCTGAAGAAGTCCTG -3' F2=5'-GTACCCTTGTACAGGTGATGTAAT-3' R1=5'-CAAACTGAGGCACAGAGATG-3' R2=5'-CAGTGCCAACTGAGAATTTGGG-3' 94°C (5 min)+[94°C (2 min)+63°C (1 min)+72°C (1 min)]×35+72°C (10 min)	-	759+316 bp=CC 759+483 bp=TT 750+316+483 bp=CT	[47, 50, 51]
IL-10 C-592A (rs1800872)	CTPP	F1=5'-ATCCAAGACAACACTACTAAGGC-3' F2=5'-ATCCTGTGACCCCGCCTGTA-3' R1=5'-CCAGAGACTGGCTTCCTACAGG-3' R2=5'-GTCACAGTGACGTGGACAAATT-3' 94°C (5 min)+[94°C (1 min)+53°C (1 min)+72°C (1 min)]×35+72°C (5 min)	-	759+545 bp=CC 759+255 bp=AA 759+545+255=CA	[47, 50, 51]

*In house design, PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism, CTPP: Confronting two-pair primers

Statistical methods

Allelic and genotypic frequencies for each SNP were estimated by PyPop software.[19] Departures from Hardy–Weinberg equilibrium (HWE; $P < 0.05$) was calculated by the method of Guo and Thompson using the Arlequin implementation[20] accessed via PyPop.[19] A Chi-squared test was performed to evaluate ethnicity-specific differences in pairwise allelic frequencies using SAS software.[21] The population genetic indices namely; observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{IS}), and the effective number of alleles (N_e) were derived using Popgene 32 software version 1.32.[22] The polymorphism information content (PIC) was calculated using the method described by Botstein *et al.*[23]

Multidimensional scaling was constructed using Reynold's genetic distance obtained from the frequencies of cytokine gene polymorphism, using Microsoft® Excel 2000/XLSTAT©-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA). Additional data that were added to the MDS database included other Iranian populations (Tehran, Yazd, Sistani and Baloch)[24] and non-Iranians[25,26]

RESULTS

Allelic and genotypic frequencies for each locus of every ethnic group are presented in Table 2. Most loci were in HWE with the exception of the following loci: IL-4-590, IL-8-251, and IL-10-819 in Fars, and IL-4-590, IL-6-174, IL-10-1082 and TNF-α-308 in Turks.

Pairwise assessment of allelic frequencies, for the 10 SNPs in our six different ethnic groups, detected statistically significant differences exclusively at the IL-4-590 locus in Gilakis versus Kurds and Lurs ($P = 0.028$ and $P = 0.022$, respectively) [Table 3]. A poorly significant difference was also observed in Gilakis versus Fars and Turk for this locus ($P = 0.082$ and $P = 0.071$, respectively). Gilakis also demonstrated this trend in other loci, namely for IL-6-174, IL-10-819 versus Fars ($P = 0.064$) and Kurds ($P = 0.072$), respectively. In addition to Gilakis, Mazanis also manifested borderline statistical differences with Kurds and Turks for IL-10-1082 ($P = 0.073$ and $P = 0.056$, respectively) [Table 3].

The values obtained for genetic diversity, namely H_o, H_e, N_e PIC, and F_{IS} are presented in Table 4. The H_o in all ethnic groups at the IL-4-590 locus was higher than expected ($H_o > H_e$, negative F_{IS} value). The widest range of N_e values was observed in Kurds ranging from 1.08 (TNF-α-308) to 2.0 (IL-2-384). In contrast, a narrower range was observed in Lurs, ranging from 1.26 (TNF-α-308) to 1.99 (IL-1β-511 and IL-10-1082). According to the criteria of Botstein *et al.*,[23] our population is characterized by a medium genetic diversity at every locus ($0.25 < \text{PIC} < 0.50$) [Table 4]. Mazanis showed excess heterozygosity (negative F_{IS} value) at all loci with the exception of the IL-10 (-592, -819, -1082). On the contrary, Gilakis showed low heterozygosity (positive F_{IS} value) at all loci, with the exception of IL-10-819 and IL-4-590, IL-2-384.

The mean values of H_o, H_e and N_e for each ethnic group are shown in Table 5. Mazanis displayed the highest mean H_o (0.50 ± 0.23), whereas Gilakis possessed the lowest (0.33 ± 0.15). The highest and lowest mean number of effective alleles (N_e) were seen in Lurs ($N_e = 1.83 \pm 0.22$) and Mazanis ($N_e = 1.64 \pm 0.27$), respectively.

The MDS plot of our data set [Figure 2a, stress 0.149] was obtained using all cytokine gene polymorphisms as shown in Table 2. This plot shows two outlier Iranian ethnic groups; the Kurds and Mazanis, at the top and bottom of the MDS plot, respectively, which were clearly separated from a tight cluster comprising the remaining ethnic groups in the center of the plot. The MDS plot of the combination of all studied Iranian populations with those of other countries was constructed using cytokine gene polymorphisms tested in all groups, (IL-1β-511, IL-2-384, IL-4-590, IL-10-1082,-819,-592 and TNF-α-308) [Figure 2b; stress = 0.096]. This MDS plot identified four distinct clusters. The European cluster included Brazil and India, to the left, which were characterized by higher frequency of IL-1β-511C, IL-10-1082G and IL-10-592A (data not shown). The Iranian population cluster, to the right, identified the Mazani ethnic group as an outlier and overlapped the HapMap-reported European data. The East Asian populations segregated into two separate clusters with Taiwan and Korea (characterized by higher occurrence of IL-4-590T and IL-10-819T; data not shown), and Japan and China, which unexpectedly shared a similar overall frequency profile with Sub-Sahara Africans.

DISCUSSION

Variations in cytokine gene polymorphisms, associated with ethnic differences, may reflect susceptibility to various diseases and has also been used as a tool for anthropological studies.[4,8,10-13,27] Such studies are of paramount importance for better diagnosis, prognosis and management of disease.[28-30]

In this study, we have evaluated the distribution of functional SNPs of ten different pro- and anti-inflammatory cytokines in six major Iranian ethnic groups. HWE analysis identified deviations in IL-4-590, IL-8-251, IL-10-1082 and TNF-α-308 loci, which may be a consequence of several reasons, namely; technical complications (sample mishandling, DNA contamination, and typing error), admixture of ethnicities, natural selection, and inbreeding. Most populations in our dataset were in HWE and supported previous findings.[2,31-33] Fars and Turk ethnicities, however, showed HWE deviation in certain loci. The accuracy of our technical procedures were confirmed by random sequencing and blinded cross-checks. Our sampling was performed in Tehran and subjects whose parents were of different ethnicities were excluded. Nonetheless, the observed deviations could be either population-specific,[31] or a consequence of high level of migration of other ethnicities (nonFars) toward Tehran (Fars) in the recent decades.[34] The latter could have caused admixture of ethnicities at the level of grand parental generations.

Allelic frequency distribution suggested a relatively high degree of homogeneity amongst our different ethnic populations. The most noticeable and significant differences were observed for the IL-4-590C allele

Table 2: Allelic and genotypic frequencies of cytokine gene polymorphisms in different ethnic groups

Cytokine	Position	Allele/Genotype	Number (%)								
			Total	Female	Male	Fars	Gilak	Kurd	Lur	Mazani	Turk
IL-1β	**−511**	C	445 (53.61)	392 (54.75)	295 (53.83)	214 (54.04)	31 (48.44)	18 (64.29)	24 (52.17)	10 (55.56)	148 (53.24)
		T	385 (46.39)	324 (45.25)	253 (46.17)	182 (45.96)	33 (51.56)	10 (35.71)	22 (47.83)	8 (44.44)	130 (46.76)
		CC	116 (27.90)	109 (30.40)	79 (28.80)	57 (28.80)	10 (31.30)	6 (42.90)	7 (30.40)	2 (22.20)	34 (24.50)
		CT	213 (51.30)	174 (48.60)	137 (50.00)	100 (50.50)	11 (34.40)	6 (42.90)	10 (43.50)	6 (66.70)	80 (57.60)
		TT	86 (20.80)	75 (20.90)	58 (21.20)	41 (20.70)	11 (34.40)	2 (14.30)	6 (26.10)	1 (11.10)	25 (18.00)
		Total	**415**	**358**	**274**	**198**	**32**	**14**	**23**	**9**	**139**
IL-1β	**−31**	T	490 (58.06)	457 (59.20)	333 (59.68)	230 (57.79)	38 (55.88)	22 (68.75)	25 (54.35)	13 (65.00)	162 (57.86)
		C	354 (41.94)	315 (40.80)	225 (40.32)	168 (42.21)	30 (44.12)	10 (31.25)	21 (45.65)	7 (35.00)	118 (42.14)
		TT	144 (34.10)	139 (36.00)	103 (36.90)	66 (33.20)	13 (38.20)	8 (50.00)	7 (30.40)	4 (40.00)	46 (32.90)
		TC	202 (47.90)	179 (46.40)	127 (45.50)	98 (49.20)	12 (35.30)	6 (37.50)	11 (47.80)	5 (50.00)	70 (50.00)
		CC	76 (18.00)	68 (17.60)	49 (17.60)	35 (17.60)	9 (26.50)	2 (12.50)	5 (21.70)	1 (10.00)	24 (17.10)
		Total	**422**	**386**	**279**	**199**	**34**	**16**	**23**	**10**	**140**
IL-2	**−384**	G	371 (50.41)	304 (53.15)	223 (46.27)	174 (50.88)	30 (55.56)	15 (50.00)	18 (45.00)	13 (65.00)	121 (48.40)
		T	365 (49.59)	268 (46.85)	259 (53.73)	168 (49.12)	24 (44.44)	15 (50.00)	22 (55.00)	7 (35.00)	129 (51.60)
		GG	102 (27.70)	84 (29.40)	57 (23.70)	49 (28.70)	8 (29.60)	4 (26.70)	6 (30.00)	4 (40.00)	31 (24.80)
		GT	167 (45.40)	136 (47.60)	109 (45.20)	76 (44.40)	14 (51.90)	7 (46.70)	6 (30.00)	5 (50.00)	59 (47.20)
		TT	99 (26.90)	66 (23.10)	75 (31.10)	46 (26.90)	5 (18.50)	4 (26.70)	8 (40.00)	1 (10.00)	35 (28.00)
		Total	**368**	**286**	**241**	**171**	**27**	**15**	**20**	**10**	**125**
IL-4	**−589**	C	544 (77.49)	457 (79.90)	348 (78.38)	260 (77.84)	53 (88.33)	16 (66.67)	29 (69.05)	13 (72.22)	173 (77.23)
		T	158 (22.51)	115 (20.10)	96 (21.62)	74 (22.16)	7 (11.67)	8 (33.33)	13 (30.95)	5 (27.78)	51 (22.77)
		CC	198 (56.40)	176 (61.50)	129 (58.10)	95 (56.90)	23 (76.70)	4 (33.30)	9 (42.90)	4 (44.40)	63 (56.30)
		CT	148 (42.20)	105 (36.70)	90 (40.50)	70 (41.90)	7 (23.30)	8 (66.70)	11 (52.40)	5 (55.60)	47 (42.00)
		TT	5 (1.40)	5 (1.70)	3 (1.40)	2 (1.20)	0 (0.00)	0 (0.00)	1 (4.80)	0 (0.00)	2 (1.80)
		Total	**351**	**286**	**222**	**167****	**30**	**12**	**21**	**9**	**112***
IL-6	**−174**	G	472 (81.38)	346 (80.84)	300 (81.08)	238 (83.22)	25 (69.44)	13 (72.22)	21 (80.77)	8 (80.00)	167 (81.86)
		C	108 (18.62)	82 (19.16)	70 (18.92)	48 (16.78)	11 (30.56)	5 (27.78)	5 (19.23)	2 (20.00)	37 (18.14)
		GG	199 (65.10)	141 (65.90)	127 (68.60)	100 (69.90)	10 (55.60)	5 (55.60)	9 (69.20)	3 (60.00)	72 (70.60)
		GC	74 (29.80)	64 (29.90)	46 (24.90)	38 (26.60)	5 (27.80)	3 (33.30)	3 (23.10)	2 (40.00)	23 (22.50)
		CC	17 (5.10)	9 (4.20)	12 (6.50)	5 (3.50)	3 (16.70)	1 (11.10)	1 (7.70)	0 (0.00)	7 (6.90)
		Total	**290**	**214**	**185**	**143**	**18**	**9**	**13**	**5**	**102***
IL-8	**−251**	T	506 (60.24)	454 (61.85)	330 (60.66)	240 (60.61)	44 (66.67)	19 (59.38)	26 (56.52)	11 (55.00)	166 (59.29)
		A	334 (39.76)	280 (38.15)	214 (39.34)	156 (39.39)	22 (33.33)	13 (40.63)	20 (43.48)	9 (45.00)	114 (40.71)
		TT	164 (39.00)	154 (42.00)	105 (38.60)	82 (41.40)	15 (45.50)	7 (43.80)	7 (30.40)	2 (20.00)	51 (36.40)
		TA	178 (42.40)	146 (39.80)	120 (44.10)	76 (38.40)	14 (42.40)	5 (31.30)	12 (52.20)	7 (70.00)	64 (45.70)
		AA	78 (18.60)	67 (18.30)	47 (17.30)	40 (20.20)	4 (12.10)	4 (25.00)	4 (17.40)	1 (10.00)	25 (17.90)
		Total	**420**	**367**	**272**	**198****	**33**	**16**	**23**	**10**	**140**
IL-10	**−1082**	G	317 (38.75)	257 (37.57)	214 (40.23)	147 (38.08)	26 (40.63)	8 (30.77)	22 (47.83)	12 (60.00)	102 (36.96)
		A	501 (61.25)	427 (62.43)	318 (59.77)	239 (61.92)	38 (59.38)	18 (69.23)	24 (52.17)	8 (40.00)	174 (63.04)
		GG	51 (12.50)	32 (9.40)	41 (15.40)	23 (11.90)	6 (18.80)	1 (7.70)	4 (17.40)	4 (40.00)	13 (9.40)
		GA	215 (52.60)	193 (56.40)	132 (49.60)	101 (52.30)	14 (43.80)	6 (46.20)	14 (60.90)	4 (40.00)	76 (55.10)
		AA	143 (35.00)	117 (43.20)	93 (35.00)	69 (35.80)	12 (37.50)	6 (46.20)	5 (21.70)	2 (20.00)	49 (35.50)
		Total	**409**	**342**	**266**	**193**	**32**	**13**	**23**	**10**	**138***
IL-10	**−819**	C	469 (71.28)	451 (72.74)	314 (69.78)	230 (71.88)	44 (78.57)	11 (55.00)	19 (63.33)	12 (75.00)	153 (70.83)
		T	189 (28.72)	169 (27.26)	136 (30.22)	90 (28.13)	12 (21.43)	9 (45.00)	11 (36.67)	4 (25.00)	63 (29.17)
		CC	164 (49.80)	160 (51.60)	106 (47.10)	79 (49.40)	17 (60.70)	3 (30.00)	5 (33.30)	5 (62.50)	55 (50.90)
		CT	141 (42.90)	131 (42.30)	102 (45.30)	72 (45.00)	10 (35.70)	5 (50.00)	9 (60.00)	2 (25.00)	43 (39.80)
		TT	24 (7.30)	19 (6.10)	17 (7.60)	9 (5.60)	1 (3.60)	2 (20.00)	1 (6.70)	1 (12.50)	10 (9.30)
		Total	**329**	**310**	**225**	**160*****	**28**	**10**	**15**	**8**	**108**
IL-10	**−592**	C	541 (73.31)	508 (73.62)	345 (71.58)	264 (75.43)	51 (79.69)	16 (61.54)	30 (71.43)	13 (81.25)	167 (69.58)
		A	197 (26.69)	182 (26.38)	137 (28.42)	86 (24.57)	13 (20.31)	10 (38.46)	12 (28.57)	3 (18.75)	73 (30.42)
		CC	202 (54.70)	187 (54.20)	126 (52.30)	97 (55.40)	21 (65.60)	6 (46.20)	11 (52.40)	6 (75.00)	61 (50.80)
		CA	137 (37.10)	134 (38.80)	93 (38.60)	70 (40.00)	9 (28.10)	4 (30.80)	8 (38.10)	1 (12.50)	45 (37.50)
		AA	30 8.10)	24 (7.00)	22 (9.10)	8 (4.60)	2 (6.30)	3 (23.10)	2 (9.50)	1 (12.50)	14 (11.70)
		Total	**369**	**345**	**241**	**175**	**32**	**13**	**21**	**8**	**120**

Contd...

Table 2: Contd...

Cytokine	Position	Allele/ Genotype	Number (%)								
			Total	Female	Male	Fars	Gilak	Kurd	Lur	Mazani	Turk
TNF-α	**−308**	A	44 (6.38)	38 (6.27)	31 (6.60)	18 (5.29)	5 (10.00)	1 (4.17)	4 (11.76)	1 (6.25)	15 (6.64)
		GG	305 (88.40)	268 (88.40)	208 (88.50)	152 (89.40)	21 (84.00)	11 (91.70)	14 (82.40)	7 (87.50)	100 (88.50)
		GA	36 (10.40)	32 (10.60)	23 (9.80)	18 (10.60)	3 (12.00)	1 (8.30)	2 (11.80)	1 (12.50)	11 (9.70)
		AA	4 (1.20)	3 (1.00)	4 (1.70)	0 (0.00)	1 (4.00)	0 (0.00)	1 (5.90)	0 (0.00)	2 (1.80)
		Total	**345**	**303**	**235**	**170**	**25**	**12**	**17**	**8**	**113***

*HWE<0.05, **HWE<0.01, ***HWE<0.001

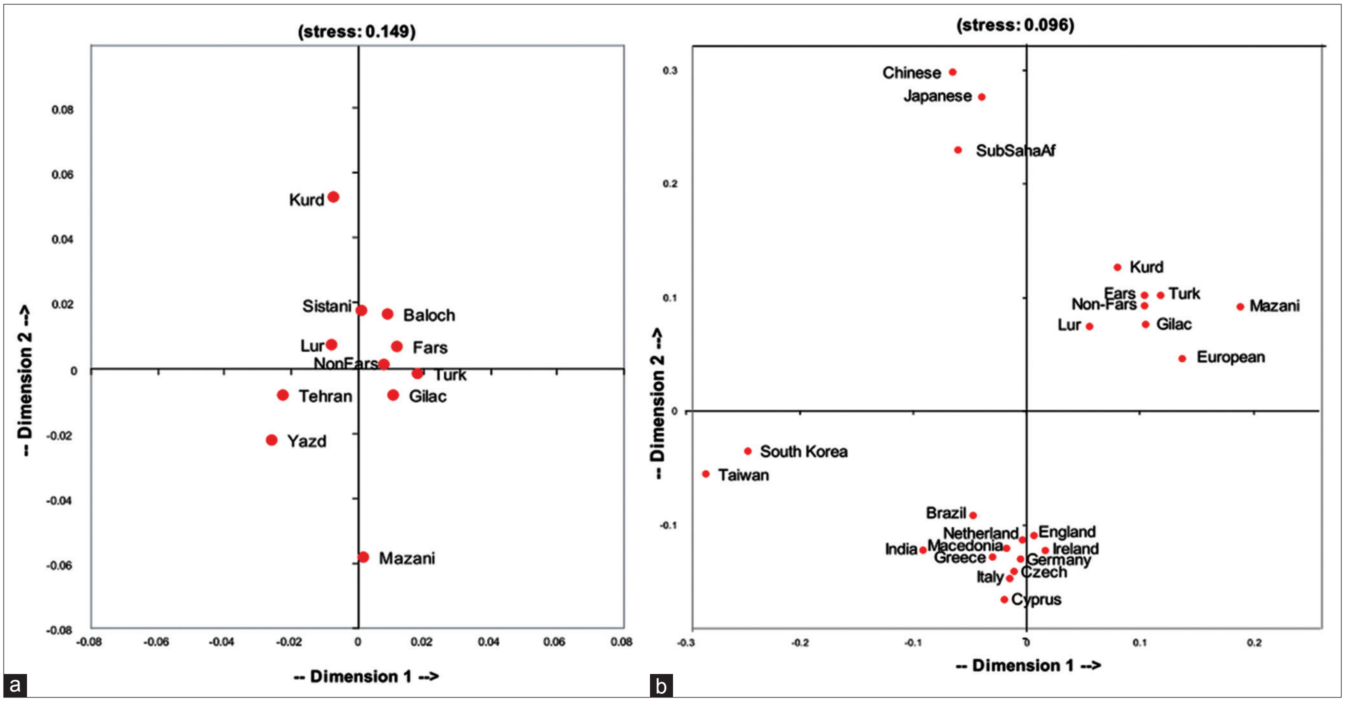

Figure 2: The multidimensional scaling (MDS) plot of cytokine gene polymorphisms in (a) Iranian populations for IL-1β (-511), IL-4 (-590), IL-6 (-174), IL-10 (-1082), IL-10 (-819), IL-10 (-592) and TNF-α (-308) loci; (b) in comparison with other populations (European, East Asia, and sub-Sahara African) for IL-1β (-511), IL-2 (-384), IL-4 (-590), IL-10 (-1082), IL-10 (-819, -592) and TNF-α (-308) loci

frequency in Gilakis (as the highest; 88%) versus Kurds and Lurs (as the lowest; 66–69%). Taking into account that IL-4-590C is associated with lower levels of immunoglobulin E production,[35] we can speculate that higher rates of Helminths infection in the north of Iran, and Gilan in particular[36] may have caused a pressure over many generations to select for genetically-based high-IL-4 producers and hence for those better equipped for IgE-mediated anti-parasitic responses.[37,38]

On another note, significant differences in allele frequencies between different ethnic groups are also caused by unequal allele frequencies in the ancestral populations.[39] With this perspective in mind, it is believed that Gilakis have originated from the South Caucasus and significantly differ from Kurds and Lurs who have distinctive origins.[40]

Our observed N_e and PIC values for TNF-α-308 indicated a low level of genetic diversity amongst Fars, Kurds and Mazanis ethnicities. In addition, the F_{IS} value observed for the examined locus suggests a condition of equilibrium in the population which is supported by the χ^2 test results used to verify the HWE. Deviation of genotypic frequencies F_{IS} and H_o in Mazani and Fars ethnic groups indicated high genetic variation. Mazani and Gilaki, as mentioned above, originated from the Caucasus and now live in the north of Iran.[40] Intriguingly, and calling for further analysis, is the highest and the lowest gene heterozygosity seen in Mazanis and Gilakis, respectively.

Ultimately, we used MDS to visualize similarities within and between Iranian ethnic groups and with those of other populations. According to MDS analysis all our ethnic groups, except for the Kurds and Mazanis, clustered together. This observation was expected for the Kurds, as they are believed to have remained isolated over the years.[40] On the other hand, Mazanis may have avoided admixture of populations

Table 3: Statistical differences between allelic frequencies of different loci among six Iranian ethnic groups

Locus	Ethnicity	Fars	Gilak	Kurd	Lur	Mazani	Turk
IL-1β -511	Fars		0.42	0.33	0.87	1.00	0.87
	Gilak			0.18	0.84	0.79	0.49
	Kurd				0.34	0.75	0.32
	Lur					1.00	1.00
	Mazani						1.00
	Turk						
IL-1β -31	Fars		0.79	0.26	0.75	0.64	1.00
	Gilak			0.27	1.00	0.60	0.78
	Kurd				0.24	1.00	0.26
	Lur					0.58	0.74
	Mazani						0.64
	Turk						
IL-2-384	Fars		0.56	1.00	0.50	0.25	0.56
	Gilak			0.65	0.40	0.59	0.37
	Kurd				0.81	0.38	1.00
	Lur					0.17	0.73
	Mazani						0.17
	Turk						
IL-4-590	Fars		*0.08*	0.21	0.24	0.56	0.91
	Gilak			**0.02**	**0.02**	0.13	*0.07*
	Kurd				1.00	0.74	0.31
	Lur					1.00	0.32
	Mazani						0.57
	Turk						
IL-6-174	Fars		*0.06*	0.21	0.78	0.67	0.71
	Gilak			1.00	0.38	0.70	0.11
	Kurd				0.71	1.00	0.34
	Lur					1.00	1.00
	Mazani						1.00
	Turk						
IL-8-251	Fars		0.41	1.00	0.63	0.64	0.75
	Gilak			0.50	0.32	0.42	0.32
	Kurd				0.82	0.78	1.00
	Lur					1.00	0.74
	Mazani						0.81
	Turk						
IL-10-1082	Fars		0.78	0.53	0.20	0.60	0.80
	Gilak			0.47	0.55	0.19	0.66
	Kurd				0.21	*0.07*	0.67
	Lur					0.42	0.19
	Mazani						*0.05*
	Turk						
IL-10-819	Fars		0.33	0.12	0.39	1.00	0.84
	Gilak			*0.07*	0.20	0.74	0.31
	Kurd				0.57	0.30	0.20
	Lur					0.52	0.40
	Mazani						1.00
	Turk						
IL-10-592	Fars		0.52	0.15	0.57	0.77	0.13
	Gilak			0.10	0.35	1.00	0.12
	Kurd				0.43	0.30	0.38
	Lur					0.52	0.85
	Mazani						0.40
	Turk						
TNF-α-308	Fars		0.19	1.00	0.12	0.59	0.58
	Gilak			0.65	1.00	1.00	0.37
	Kurd				0.39	1.00	1.00
	Lur					1.00	0.28
	Mazani						1.00
	Turk						

$P<0.05$ are bolded and those <0.1 are italicized

Table 4: Calculated genetic diversity values for the different Iranian ethnicities

Locus	Population	Diversity parameter				
		H_o	H_e	N_e	PIC	F_{IS}
IL-1β -511	**Total**	**0.50**	**0.49**	**1.99**	**0.49**	**−0.01**
	Fars	0.50	0.49	1.98	0.50	−0.01
	Gilak	0.34	0.50	1.99	0.50	0.31
	Kurd	0.42	0.47	1.84	0.41	0.06
	Lur	0.43	0.51	1.99	0.49	0.12
	Mazani	0.66	0.52	1.97	0.47	−0.35
	Turk	0.57	0.49	1.99	0.50	−0.15
IL-1β -31	**Total**	**0.47**	**0.48**	**1.95**	**0.48**	**0.01**
	Fars	0.49	0.49	1.95	0.49	0.49
	Gilak	0.35	0.50	1.97	0.50	0.28
	Kurd	0.37	0.44	1.75	0.41	0.12
	Lur	0.47	0.50	1.98	0.49	0.03
	Mazani	0.50	0.47	1.83	0.47	−0.09
	Turk	0.50	0.48	1.94	0.49	−0.03
IL-2-384	**Total**	**0.45**	**0.50**	**1.99**	**0.50**	**0.08**
	Fars	0.44	0.50	1.99	0.50	0.11
	Gilak	0.51	0.50	1.97	0.50	−0.05
	Kurd	0.46	0.51	2.00	0.46	0.50
	Lur	0.30	0.50	1.98	0.50	0.39
	Mazani	0.50	0.47	1.83	0.38	−0.09
	Turk	0.47	0.50	1.99	0.50	0.05
IL-4-590	**Total**	**0.42**	**0.35**	**1.53**	**0.35**	**−0.21**
	Fars	0.41	0.34	1.52	0.36	−0.21
	Gilak	0.23	0.23	1.25	0.26	−0.13
	Kurd	0.66	0.46	1.80	0.33	−0.50
	Lur	0.52	0.43	1.74	0.54	−0.22
	Mazani	0.55	0.42	1.67	0.47	−0.38
	Turk	0.41	0.35	1.54	0.65	−0.19
IL-6-174	**Total**	**0.25**	**0.30**	**1.42**	**0.31**	**0.13**
	Fars	0.26	0.28	1.38	0.26	0.04
	Gilak	0.27	0.27	1.73	0.43	0.27
	Kurd	0.33	0.42	1.67	0.41	0.40
	Lur	0.23	0.32	1.45	0.30	0.25
	Mazani	0.40	0.35	1.47	0.38	−0.25
	Turk	0.22	0.29	1.42	0.30	0.24
IL-8-251	**Total**	**0.43**	**0.47**	**1.91**	**0.48**	**0.09**
	Fars	0.38	0.47	1.91	0.47	0.19
	Gilak	0.42	0.45	1.80	0.36	0.04
	Kurd	0.31	0.49	1.93	0.33	0.35
	Lur	0.52	0.50	1.96	0.50	−0.06
	Mazani	0.70	0.52	1.98	0.47	−0.41
	Turk	0.45	0.48	1.93	0.48	0.05
IL-10 -1082	**Total**	**0.52**	**0.47**	**1.91**	**0.47**	**-0.10**

Contd...

Table 4: Contd...

Locus	Population	Diversity parameter				
		H_o	H_e	N_e	PIC	F_{IS}
	Fars	0.52	0.47	1.89	0.45	−0.10
	Gilak	0.43	0.49	1.93	0.47	0.09
	Kurd	0.46	0.44	1.74	0.41	−0.08
	Lur	0.60	0.51	1.99	0.49	−0.21
	Mazani	0.40	0.50	1.92	0.47	0.16
	Turk	0.55	0.46	1.87	0.46	−0.18
IL-10-819	**Total**	**0.42**	**0.40**	**1.69**	**0.41**	**−0.05**
	Fars	0.45	0.40	1.67	0.41	−0.11
	Gilak	0.35	0.34	1.50	0.36	−0.06
	Kurd	0.50	0.52	1.98	0.50	−0.01
	Lur	0.60	0.48	1.86	0.46	−0.29
	Mazani	0.25	0.40	1.60	0.21	0.33
	Turk	0.39	0.41	1.70	0.59	0.03
IL-10-592	**Total**	**0.37**	**0.39**	**1.64**	**0.39**	**0.04**
	Fars	0.40	0.37	1.58	0.39	−0.07
	Gilak	0.28	0.32	1.47	0.40	0.13
	Kurd	0.30	0.49	1.89	0.49	0.35
	Lur	0.38	0.41	1.68	0.46	0.06
	Mazani	0.12	0.32	1.43	0.21	0.58
	Turk	0.37	0.42	1.73	0.43	0.11
TNF-α-308	**Total**	**0.10**	**0.11**	**1.13**	**0.12**	**0.12**
	Fars	0.10	0.10	1.11	0.13	−0.05
	Gilak	0.12	0.18	1.21	0.26	0.33
	Kurd	0.08	0.08	1.08	0.13	−0.04
	Lur	0.11	0.21	1.26	0.30	0.43
	Mazani	0.12	0.12	1.13	0.21	−0.06
	Turk	0.09	0.12	1.14	0.31	0.21

H_o: Observed heterozygosity, H_e: Expected heterozygosity, N_e: Effective number of alleles, PIC: Polymorphism information content, F_{IS}: Fixation index

Table 5: Mean values of observed and expected heterozygosities and number of effective alleles at all loci

Ethnicity	Observed	Expected	N_e
Fars	0.40±0.12	0.39±0.11	1.70±0.28
Gilak	0.33±0.15	0.41±0.09	1.70±0.25
Kurd	0.38±0.13	0.41±0.11	1.67±0.24
Lur	0.43±0.15	0.46±0.08	1.83±0.22
Mazani	0.50±0.23	0.42±0.13	1.64±0.27
Turk	0.40±0.11	0.40±0.10	1.72±0.26

following Russian invasion of north of Iran in the early 18th century[41] and have remained secluded to the present days.

The three clusters observed in Figure 2b are in good agreement with a long history of migration and separation. While the distribution of cytokine alleles clearly distinguishes Iranian groups from most European groups, it maintains a close genetic affinity with this group. Accordingly, it has been suggested that the Iranians might have relatively close evolutionary history with people of Russia rather than East Asian populations.[42] Despite the clear clustering of all Asian groups from other groups, the position of the Sub-Saharan Africans among Chinese and Japanese populations remains intriguing.

In general, our data, based on cytokine gene polymorphism, mostly indicate genetic homogeneity of the Iranian population, despite its multi-ethnic composition. The allelic and genotypic frequencies of Iranian populations present closer affinity to Europeans rather than to Asians groups. The few mentioned disparities in cytokine allele frequencies for some of our ethnic populations, however, call for careful selection of cases and controls and subsequent adjustments when performing disease association studies, to avoid misrepresentations.

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